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Benthic Fauna Colonization of a New Reservoir with Particular Reference to the Chironomidae

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At the time of first filling of Laurel Creek Reservoir in the spring of 1967 the benthic fauna consisted of submerged terrestrial organisms and obligate and facultative rheophilic species. Two months after initial filling substantial populations of colonizing limnophilic species developed in the habitat whereas the terrestrial and obligate rheophilic components were lost. By the 4th month the fauna was dominated by euryoxybiontic limnophiles and by facultative species. In the period immediately prior to reservoir drainage in the autumn of 1967 the population densities of the euryoxybiontic chironomids declined whereas polyoxybiontic species continued to increase in abundance. These changes in the dominance hierarchy of the chironomid fauna were associated with the partial loss of the rich deposits of organic debris by siltation and decomposition. Survival of winter drainage of the reservoir by many limnophilic species produced an appreciably different pattern of colonization when the reservoir refilled in 1968. During the second summer of the reservoir's existence most euryoxybiontic chironomids further declined in abundance and were replaced by polyoxybiontic forms as the dominant species. The facultative species originally derived from the creek fauna are adapted to a wide range of environmental conditions and the modifications of the reservoir habitat with time had little effect on their abundance.

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INTRODUCTION

ARTIFICIAL IMPOUNDMENTS are becoming an increasingly important component of the surface water resource of this continent. The wide range of edaphic, climatic, and morphological characteristics of such impoundments would result in marked differences in the extent and nature of the biological communities that develop in the newly constructed habitats. Many authors (reviewed by Weerekoon, 1956) have attempted to classify natural lakes on the basis of their bottom faunas. The complex interactions, however, of the numerous factors affecting the composition and abundance of the benthos has raised certain questions as to the validity of such classifications (Brundin, 1949; Weerekoon, 1956). Brundin (1958) later modified the bottom faunistical lake type system and developed a more refined classification although a clear understanding of the factors producing a change from one lake type to another is still required.

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The study of the development of the benthic regime in a wide variety of newly formed reservoirs may offer a potential source of insight into the influence of certain factors on the composition and abundance of the benthos. In an arid region, or where the reservoir has been cleared of organic debris, autochthonous and allochthonous production can produce a change from oligotrophy to eutrophy as typified by the benthic fauna. Conversely, a reservoir that possesses many of the physical characteristics of an oligotrophic habitat can display an early eutrophic stage due to the quantities of organic debris present in the reservoir basin prior to impoundment. This latter sequence was found to occur in a mountain reservoir in Alberta by Nursall (1952) and Fillion (1967).

Although there are thought to be certain advantages to the study of the development of the benthic regime of new reservoirs, this field of limnology has received little attention, particularly in North America. There have been many investigations on older reservoirs but only very limited studies from the time the impoundment is first filled. Exceptions are to be found in the work of Burris (1954) and Nursall (1952).

The Russian literature dealing with the processes of colonization and stabilization of the fauna and flora of newly constructed reservoirs is more extensive than that of North America. Ozhegova (1962) has studied the complete pattern of colonization, and Melnikov and Lubyanov (1958) and Yaroshenko and Naberezhnyi (1959) have studied the benthos in addition to the zooplankton. Studies on the development of the macrobenthos community have been conducted by Sokolova (1963) and Morduchai-Boltovskoi (1961).

The emphasis in the Russian work has been on the large fluviatile reservoirs constructed on the larger rivers. These impoundments are not directly comparable with many of the smaller ones studied in North America as they commonly have appreciable currents passing along the length of the basin.

The present study deals with the rate and extent of development of the macrobenthos community of Laurel Creek Reservoir during the first two summers after initial filling. The marginal fauna of the reservoir (Paterson and Fernando, 1969a) and the effect of winter drainage on the benthic fauna (Paterson and Fernando, 1969b) have been previously considered.

LAUREL CREEK RESERVOIR

Laurel Creek Reservoir has been described by Paterson and Fernando (1969c). It is a small (64.75 ha), shallow (maximum depth 3.6 m, mean depth 0.94 m) impoundment, located 1 km northwest of the city of Waterloo, Ont. Prior to impoundment the site consisted of areas of unused pasture, which supported extensive growths of grasses and weeds, and areas that had been forested (Fig. 1). The trees were removed but forest debris, leaf litter, and ash from the burning of the logs covered large areas of the substrate. No effort was made to remove any of the stumps prior to the time that the reservoir was first filled. In one small area the grass overburden was removed to expose a clay substrate. The reservoir site is well exposed to the prevailing winds.

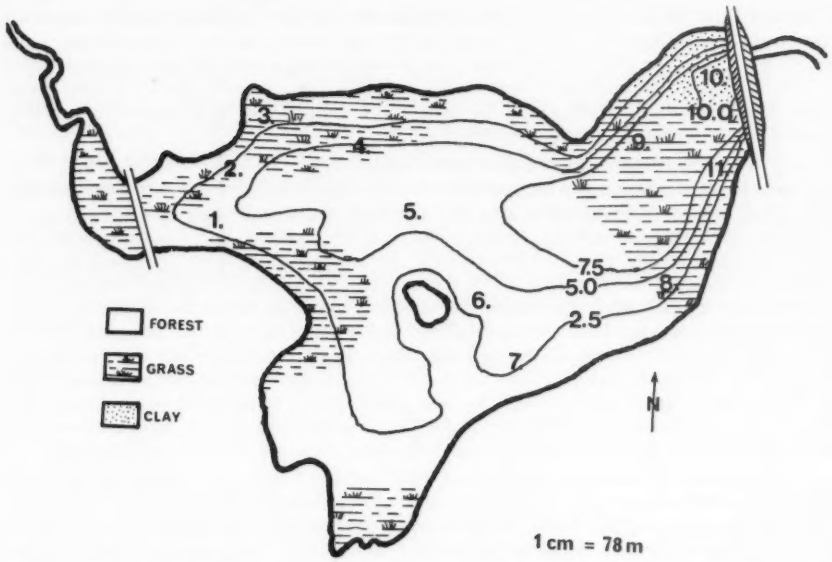

FIG. 1. Laurel Creek Reservoir showing location of benthic sampling stations and preimpoundment distribution of substrate components. Contours are in feet at full supply level.

The reservoir first began to fill in late March of 1967 and full supply level was reached on March 31, 1967. A lowering of the water level began in mid-September and drainage was complete by October 10, 1967. Refilling began in mid-March of 1968 and full supply level was achieved on March 31 of that year. The reservoir was again drained in December of 1968 and was refilled for the third time in early April of 1969. Although the reservoir is small the low summer flow rates in Laurel Creek resulted in an average retention time of 63 days.

METHODS

Certain physical and chemical parameters were determined at 2-week intervals in Laurel Creek immediately upstream and at three sites along the length of the reservoir. Samples were collected from the surface layer except at the deepest point in the reservoir (3.6 m) where sampling was done at the surface and at a depth of 3.0 m. Dissolved oxygen was determined by the unmodified Winkler method. The *p*H was measured with a Beckman model N *p*H meter. Conductivity measurements were at 20 C with a Radiometer C.D.M. conductivity meter. Water temperature was measured with a Y.S.I. oxygen and temperature meter.

Fillion (1967) suggests that the amount of sedimentation that occurs in a given area of a reservoir influences the abundance of the benthic fauna. To test this hypothesis in Laurel Creek Reservoir a simple apparatus was used to measure the rate of sedimentation. This consisted of a 6-inch square plate of 0.5-inch thick iron onto which was welded a 4-inch piece of pipe. This pipe had an inside diameter of 3¼ inch and would snugly seat a wide-mouthed 16-oz bottle. Two opposing holes in the upper edge of the pipe served to anchor a wire loop that was in turn attached to a rope leading to a buoy at the surface. This design was heavy enough to insure even settling without tipping. Observations made by lowering the sampler rapidly to the bottom showed that

the disturbance created when the sampler settled on the substrate did not cause extraneous sediment to be washed into the collecting bottle. Such samplers were placed at three sites along the length of the reservoir and left for 2-week intervals during the summer of 1967. Each sediment sample was treated by allowing it to settle for 2 or 3 days in an Imhoff cone. After the excess water was decanted off, the dry weight and ash weight of the sediment were determined by drying at 60 C for 48 hr and by igniting at 600 C for 2 hr.

The benthic fauna of the reservoir was examined from the time of first filling in 1967 at 11 sampling sites (Fig. 1). The first samples were obtained 2 weeks after the reservoir reached full supply level in 1967. Sampling was then continued at approximately 2-week intervals till mid-September when the reservoir began to drain. In the spring of 1968 sampling commenced again 2 weeks after the reservoir filled and was continued till early August. At each station a single sample was obtained with a 6-inch Ekman grab. This type of sampling program is inadequate to accurately determine the population density of the benthos at the individual stations but is sufficient for determining the overall pattern of colonization of the habitat (Paterson, MS, 1969). The samples were swirled with tap water and drained through a 0.3-mm nylon net. Fine silt was washed through the net with a gentle stream of water and then all material retained on the net was preserved in 5% formalin for later examination. The preserved material was examined in small portions in a counting tray under 12.5 magnifications. All chironomid larvae were mounted in A.C.S. (Edward Gurr Ltd., London) direct mounting medium and identified.

Throughout the summers of 1967 and 1968 occasional duplicate benthic samples were placed in aerated aquaria and the emerging adult chironomids identified. To overcome certain difficulties in associating adults and larvae, attempts were made to rear individual larvae. Adult chironomids were also collected along the shores of the reservoir with a sweep net and aspirator, usually during one evening period of each week that the reservoir was full.

To determine the importance of the creek macro-invertebrate community in the colonization of the reservoir the fauna was sampled by a "kick" technique similar to that used by Hynes (1961). The net used had mesh openings of 0.3 mm. From October of 1966 until the reservoir first filled in late March of 1967 at least two samples, each representing the fauna of approximately 220 cm^2 of the substrate, were collected from each of two pools and two riffles within the proposed reservoir basin plus an additional pool and riffle section upstream from the reservoir site. After the filling of the reservoir, sampling was continued at the upstream section until March of 1968. Sampling was done at 2-week intervals when ice conditions allowed. The samples were treated in the same manner as the benthic samples from the reservoir. All organisms were counted to determine the relative abundance of each species.

Throughout this paper organisms that were abundant in Laurel Creek but failed to establish permanent populations in the reservoir are considered to be obligate rheophilic species. Species common to both the creek and reservoir are termed facultative whereas species restricted to the lentic environment are considered to be limnophilic forms. It is realized that this classification has only limited general applicability as the apparent restriction of a species to either the lentic or lotic habitat is dependent on the particular conditions prevailing in the habitats studied.

RESULTS

Physico-Chemical Conditions

Certain selected physical and chemical characteristics of Laurel Creek and Laurel Creek Reservoir have been dealt with by Paterson and Fernando (1969a). The reservoir and creek are hard water, moderately alkaline environments. The summer conductivity ranged from 350 to 510 μmhos and the *p*H varied from 7.5 to 8.8.

For the first 2 months after filling of the reservoir in the spring of 1967 the temperature of the surface water, as measured at a station near the dam,

did not attain temperatures much above 10 C (Fig. 2). In early June the water warmed rapidly to temperatures in excess of 20 C, and then maintained this level until September. The seasonal pattern of temperature found during 1968 was similar although a higher summer maximum was recorded. The water temperatures at the surface are essentially the same as those prevailing at the mud–water interface and thus directly affecting the benthic fauna. On three very warm, calm days the bottom temperature at the maximum depth was 1.2–1.5 C degrees lower than that of the surface but on all other sampling dates the vertical variation was less than 0.5 degrees C. Most of the reservoir (90%) is less than 2 m deep and at these depths the maximum recorded vertical temperature difference was 0.2 degrees C. The measurement of temperature and dissolved oxygen at two other surface stations along the length of the reservoir did not reveal substantial spatial variations.

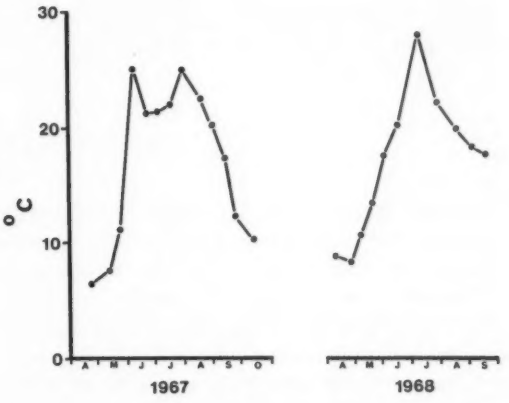

FIG. 2. Temperature of the surface water at a station located near the dam in Laurel Creek Reservoir.

The shallow depth and exposure of the reservoir basin that prevented the establishment of well-defined vertical temperature gradients also prevented extensive deoxygenation as the large quantities of organic debris decomposed. The lowest dissolved oxygen level found 0.6 m above the substrate at the deepest point was 40% of full saturation and concentrations lower than 60% were uncommon. Unfortunately, no attempt was made to determine oxygen microstratification at the mud–water interface. As shown by Brundin (1951) this can be an important factor affecting the species composition of the chironomid fauna.

SEDIMENTATION

The material collected in the sediment samplers cannot be considered to represent the true rate of sedimentation in the reservoir. Material trapped in the samplers would remain whereas that settling on the bottom would be in a near constant state of resuspension and deposition. However, the results serve to give a measure of the rate at which the organic debris of the reservoir

basin was being lost by siltation. The total amount of sediment trapped in the three sampling locations varied because of depth and the ease with which the particular substrate type could be taken into suspension. If the results from the three samplers are pooled then the accumulated average amount of sediment settling on the reservoir substrate with time can be plotted as shown in Fig. 3.

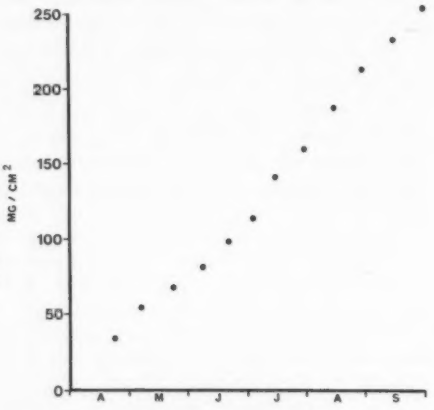

FIG. 3. Average accumulative amount of total dry weight of sediment settling on the substrate of Laurel Creek Reservoir during the summer of 1967

Although much of this apparent accumulation would be the result of resuspension the results indicate that the reservoir substrate slowly silted over during the summer of 1967. This is in keeping with observations made immediately after the reservoir was drained in October of 1967 when the organic detritus originally present on the reservoir bottom was overlain with a layer of fine sediment approximately 2 cm thick. Much of the material sedimenting on the reservoir substrate consisted of fine particles of inorganic material. Throughout the summer of 1967 volatile material composed between 13.6 and 57.1%, with an average of 31.5%, of the total dry weight of the sediment.

BENTHIC FAUNA

The construction of a small shallow impoundment rich in organic detritus resulted in an extensive colonization by a macroinvertebrate community. In 1967, 107 recognized taxa were collected from the habitat (Paterson and Fernando, 1969c). Because of the shallow nature of the reservoir much of the area can be considered to lie in the littoral zone and many of the species associated with the marginal region of the habitat were occasionally distributed throughout the reservoir. However, the benthic fauna was dominated by the chironomids and oligochaetes with the nematodes and oribatoid mites being subdominant (Table 1). The small numbers of other species collected in the benthic samples was partially a result of the strong preference of many species for the shore regions. An Ekman grab is also an inefficient sampler for large mobile species, such as adult Hemiptera and Coleoptera, and would not reveal their actual abundance in the benthos.

TABLE 1. Average monthly standing crop of macrobenthos in Laurel Creek Reservoir in 1967 and 1968 (no./m²).

	1967						1968				
	Apr.	May	June	July	Aug.	Sept.	Apr.	May	June	July	Aug.
Oligochaeta	725	900	2,677	921	7,597	7,930	909	3,243	8,062	11,924	8,434
Nematoda	32	435	168	118	0	340	47	183	164	142	125
Oribatoidea	75	34	415	74	337	587	220	190	215	297	219
Chironomidae	211	409	6,442	14,949	9,262	7,790	1,886	1,167	3,450	7,169	5,336
Others	35	104	178	194	700	647	73	49	376	630	1,134
Total	1,078	1,882	9,880	16,256	17,896	17,294	3,135	4,832	12,267	20,162	15,248

OLIGOCHAETA

The oligochaetes, most of which are referrable to undetermined species of *Limnodrilus*, were abundant in the creek fauna and invaded the reservoir at the time of first filling in 1967 (Fig. 4). The population density of this group began to increase as the water temperature of the reservoir increased in June and reached a population density approximating 8000/m^2 in August. Progressive silting-over of the organic bottom deposits of the reservoir did not have a well-defined effect on the population densities found during the latter part of the summer of 1967 as the population remained relatively constant during August and September.

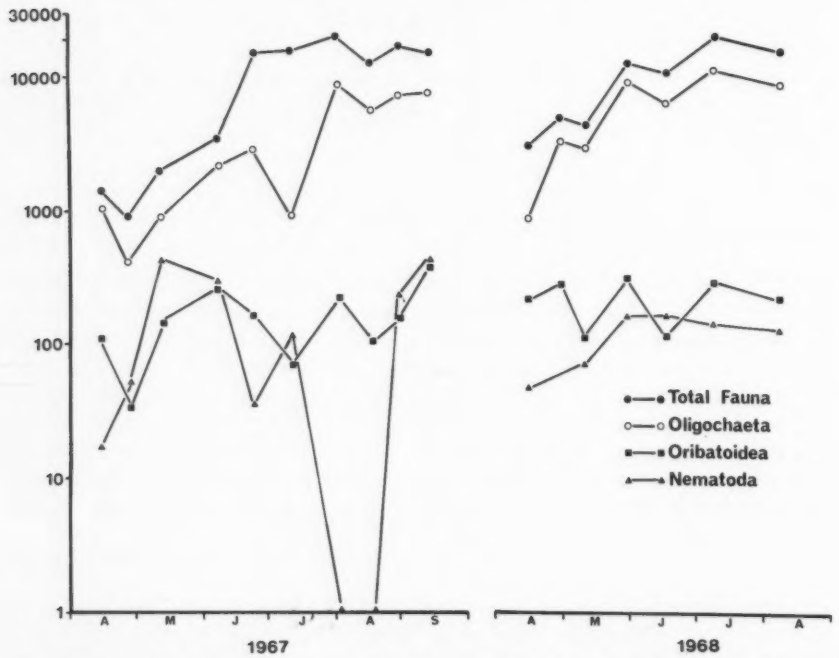

FIG. 4. Population development of the total benthic fauna and the oligochaetes, oribatoid mites, and nematodes in Laurel Creek Reservoir during 1967 and 1968. Expressed as average number/m^2.

The oligochaetes did not survive in the exposed substrate during the winter of 1967–68 (Paterson and Fernando 1969b) and the numbers reinvading the habitat from Laurel Creek at the time of filling in the spring of 1968 were very similar to the population density found in the spring of 1967. The pattern of population increase in the second summer of the reservoir's existence was essentially identical to that found in 1967. Many aquatic oligochaetes are facultative and can maintain large populations in both lotic and lentic environ-

ments. The ability to be successful in a wide variety of environmental conditions explains the similarities of the population structures during the two summers of the study in the face of changing conditions within the reservoir. As the carrying capacity of the habitat for chironomids was decreasing although the oligochaetes were not affected, the oligochaetes came to be relatively more important in the fauna of the reservoir during 1968 (Table 1).

NEMATODA

The occurrence of a nematode population in the reservoir when it was first sampled in both 1967 and 1968, coupled with their abundance in Laurel Creek, suggests that the species present were facultative forms derived from the creek fauna. The changes in population density shown in Fig. 4 are only approximate as most of the aquatic nematodes encountered in this study were very small and difficult to efficiently extract from benthic samples. The disappearance of this group on two sampling dates in August of 1967 may result from extreme sampling error as there is no other ready explanation for this type of seasonal pattern. Like the oligochaetes, the nematodes were unable to overwinter in the drained reservoir and displayed a similar pattern of colonization in 1968 although the marked fluctuations in population density were not apparent. The overall similarity in the average density maintained during the first two summers of the reservoir's existence would suggest that the nematodes are not affected by the environmental modifications of the habitat.

ORIBATOIDEA

The occurrence of an appreciable population of oribatoid mites in the benthic fauna of Laurel Creek Reservoir throughout the two summers of the study is of interest as the authors have been able to find very few published accounts of the occurrence of this group in other benthic habitats. Berg (1938) found small numbers of *Notaspis lacustris* in the littoral zone of Esrom Lake in association with large populations of several species of *Hydracarina*. In Laurel Creek Reservoir the average population density of oribatoids often reached 300/m^2 (Fig. 4) whereas the hydracarinids were never found in average densities greater than 141/m^2. The reservoir population derived from the inundated creek showed seasonal fluctuations but did not display substantial population increases within the reservoir. The average monthly population densities found during 1967 and 1968 were similar as a result of failure to overwinter and the absence of any effect of environmental modification produced by siltation.

MINOR ELEMENTS

Because there is no well-defined distinction between the benthic and marginal zones of Laurel Creek Reservoir, the Ekman grab samples were often found to contain small numbers of species, such as immature stages of Coleoptera and Hemiptera, which were abundant in the marginal zone samples (Paterson and Fernando, 1969a). In addition to this component, which included 17 recognized species in 1967 and 7 in 1968, there were a number of species

that are true members of the benthic community as recognized by their abundance and frequent occurrence. The average monthly abundance of these organisms are given in Table 2. The increase in population density of *Hyalella azteca* and *Agryalea multipunctata* in 1968 was related to the colonization of Laurel Creek Reservoir by *Potamogeton foliosus* Raf. as the samples that contained this macrophyte were the ones with large numbers of these species. The decrease in the population of *Planorbula jenksii*, and to a lesser extent the other species of gastropods, was related to the silting over of the organic detritus of the reservoir bed.

INUNDATED TERRESTRIAL ORGANISMS

When the reservoir was first filled in the spring of 1967 it was observed that numbers of terrestrial organisms, especially earthworms and tipulid larva, were common. In the grab samples they were found in numbers as high as $28/m^2$ until June 9, 1967, whereupon they disappeared from the fauna and were not found again during the course of the study. The density of this fauna, as collected by the Ekman grab, is thought to be an underestimate as there appeared to be a zone of concentration near the shore in water shallower than normally sampled.

Although the numbers per square meter were small, the standing crop, in terms of biomass, was high as they were mostly large organisms. In a reservoir, such as Laurel Creek Reservoir, there would be a standing crop of submerged terrestrial organisms that would be adequate to support a fish fauna immediately after the reservoir was filled and before the establishment of an extensive aquatic benthic fauna. In Beaver Reservoir, which lies on the White River between Arkansas and Missouri, young largemouth bass (*Micropterus salmoides*) collected near shore were found to utilize earthworms (13.0% of food volume) when the size of the reservoir increased to flood a large area of adjacent land (Applegate and Mullan, 1967).

CHIRONOMIDAE

The chironomids are among the most important components of the benthic fauna of a majority of aquatic habitats and in this respect Laurel Creek Reservoir was no exception, even during the first summer of its existence. The chironomids found in Laurel Creek Reservoir during the course of this study are given in Table 3. The nomenclature is that of Sublette and Sublette (1965) except where noted.

Procladius freemani, a species common to the creek fauna, was found in small numbers ($8/m^2$) when the reservoir was first sampled in 1967 (Fig. 5). It was then absent from the fauna, either as a result of emergence, or due to sampling error, until late June. The population colonizing the reservoir in June increased in abundance until an average standing crop of $188/m^2$ was found on the last sampling date in September. This facultative species was unable to overwinter in the exposed substrate and was absent from the benthic fauna on the first sampling date in 1968. It quickly recolonized the reservoir in May and continued to increase in abundance until the population in July

TABLE 2. The average monthly abundance (no./m²) of minor elements in the benthic fauna of Laurel Creek Reservoir in 1967 and 1968.

	1967						1968				
	Apr.	May	June	July	Aug.	Sept.	Apr.	May	June	July	Aug.
Hydra sp.	0	4	60	0	24	8	0	0	0	31	110
Helobdella stagnalis (L.)	0	0	4	0	105	8	0	0	0	31	28
Hyalella azteca (Saussure)	2	62	43	0	0	0	17	4	7	94	438
Hydracarina	9	27	24	0	0	19	0	0	62	110	141
Agraylea multipunctata Curtis	0	0	0	0	0	4	17	0	263	282	16
Dubiraphia sp.	0	0	0	0	27	2	0	4	17	16	16
Physidae (*P. sayii* Tappan, *P. integra* Haldeman, *Aplexa hypnorum* (L.)	2	0	10	86	263	67	0	0	0	0	31
Planorbula jenksii (Carpenter)	0	0	4	7	72	139	117	0	0	0	0
Helisoma anceps (Menke)	0	0	8	0	115	247	0	0	0	19	176
Ferrissia sp.	0	0	0	0	4	59	0	0	0	0	0
Pisidium compressum Prime	0	0	0	0	31	12	0	24	11	0	0
Sphaerium (*S. sulcatum* (Lamarck)), *S. transversum* (Say)	0	0	0	62	36	16	0	15	2	4	0

TABLE 3. The larval Chironomidae of Laurel Creek Reservoir in 1967 and 1968.

Tanypodinae
- *Anatopynia dyari* (Coquillett)[a]
- *Ablabesmyia mallochi* (Walley)[a]
- *Procladius freemani* Sublette[a]
- *Procladius bellus* (Loew)[a]
- *Tanypus carinatus* Sublette[a]

Diamesinae
- *Prodiamesa nr. bathyphila* Kieffer

Orthocladiinae
- *Orthocladius* spp.
- *Cricotopus trifasciatus* (Panzer)[a]
- *Trichocladius* spp.
- *Psectrocladius*? sp.
- *Metriocnemus* sp.
- *Corynoneura* (*C.*) *celeripes* Winnertz
- *C.* (*Thienemanniella*) sp.

Chironominae
Tribe Chironomini
- *Chironomus* (*C.*) *attenuatus* Walker[a]
- *C.* (*C.*) *atrella* (Townes)[a]
- *C.* (*C.*) *tentans* Fabricius[a]
- *C.* (*Cryptochironomus*) sp.
- *C.* (*C.*) *psittacinus* Meigen[a]

Chironominae (*con't*)
- *C.* (*C.*) *digitatus* Malloch[a]
- *C.* (*C.*) *abortivus* Malloch[a]
- *C.* (*C.*) *tenuicaudatus* Malloch[a]
- *C.* (*Dicrotendipes*) *nervosus* Staeger[a]
- *C.* (*Endochironomus*) *brachialis* Coquillett[a]
- *Glyptotendipes barbipes* (Staeger)[a]
- *Microtendipes pedellus* (De Geer)[a]
- *Microtendipes* sp.
- *Pseudochironomus fulviventris*? (Johannsen)
- *Paralauterborniella nigrohalteralis* (Malloch)
- *Paratendipes* sp.
- *Phaenopsectra* spp.
- *Polypedilum* (*P.*) spp.
- *P.* (*P.*) *simulans* Townes[a]
- *Stictochironomus* sp.

Tribe Tanytarsini
- *Micropsectra* spp.
- *Tanytarsus* (*Tanytarsus*) spp.
- *T.* (*Cladotanytarsus*) spp.
- *T.* (*Rheotanytarsus*) sp.
- Group "A" of Roback (1957) spp.[b]
- *Zavrelia* sp.[c]

[a]Identified on the basis of reared adults.
[b]The affinities of this larval group are obscure but they are probably closely related to *T.* (*Cladotanytarsus*).
[c]This larva keys to *Zavrelia* in Roback (1957) and is included in this genus by Sublette and Sublette (1965). In Hamilton (MS) it keys to *Stempellinella*, a genus not included in the North American fauna by Sublette and Sublette (1965).

and August was substantially greater than that of 1967. Rearing of adults revealed that very small numbers of the larvae referred to *P. freemani* are *P. bellus*. During 1967 a few individuals of *Ablabesmyia mallochi* were found in the reservoir. This species was absent in 1968 but two other Tanypodinae, *Anatopynia dyari* and *Tanypus carinatus*, were found in small numbers. These latter two species are considered to be limnophilic forms that colonized from other lentic environments while *A. mallochi* was very abundant in Laurel Creek. Limited sampling of the reservoir in 1969 suggests that *T. carinatus* is becoming codominant with *P. freemani* as the major tanypod in the reservoir.

The members of the subfamily Orthocladiinae (Fig. 5) were a major component (47.8%) of the chironomid fauna when the reservoir was first sampled in the spring of 1967. This population, which consisted of creek-derived species of *Orthocladius* spp., *Metriocnemus* sp., *Trichocladius* sp., and *Corynoneura* spp., declined rapidly until no members of this group were found during July and August. These species, with the possible exception of *Corynoneura* spp., did not establish populations in the lentic environment once the originally introduced individuals perished or emerged. The apparent reappearance of

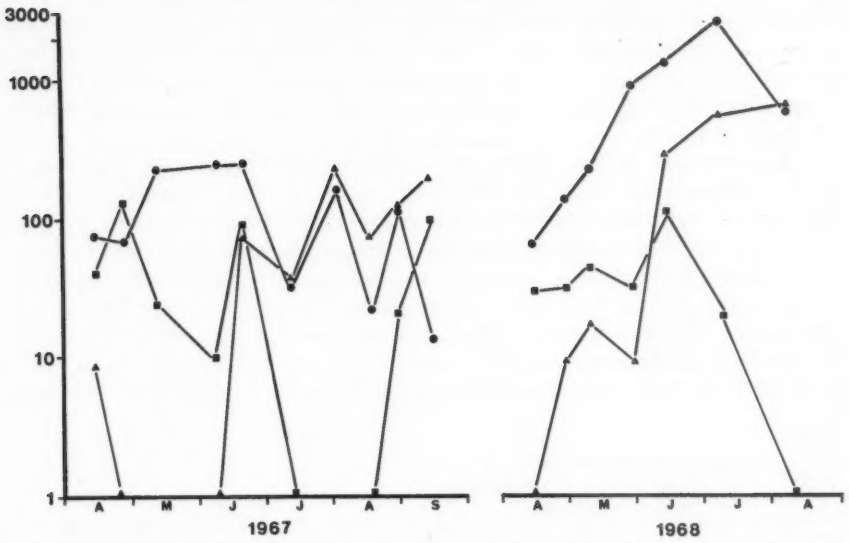

FIG. 5. Population development of *Procladius freemani* (▲—▲), Orthocladiinae (■—■), and Tanytarsini (●—●) in Laurel Creek Reservoir during 1967 and 1968. Expressed as average number/m².

an orthoclad fauna in September was the result of colonization by *Cricotopus trifasciatus*. This species is commonly found in lentic environments (Roback, 1957). When the reservoir refilled in the spring of 1968 the orthoclads were again abundant. In addition to a creek-derived assemblage similar to that found in early 1967 there were numbers of *C. trifasciatus* as a result of their ability to overwinter in the exposed substrate. The loss of an orthoclad fauna in August is similar to the conditions in 1967 where the introduced rheophilic forms are lost and *C. trifasciatus* larvae have emerged and the new generation has not been established.

After invading the reservoir from the inundated creek in the spring of 1967 the members of the tribe Tanytarsini (Fig. 5) showed several population peaks as a result of the nonsynchronized emergence and hatching of the several species present. The common members of the tribe, especially during the latter part of the summer of 1967, were *T.* (*Cladotanytarsus*) spp. and Group "A" of Roback (1957). In April, May, and June specimens of *Zavrelia* sp., *T.* (*Rheotanytarsus*) sp., *T.* (*Tanytarsus*) spp., and *Micropsectra* spp. were the dominant forms. By the time the reservoir was drained in the fall of 1967 the average standing crop of Tanytarsini was not substantially different from that found during the early part of the summer. This group did not overwinter and the population density in the spring of 1968 was very similar to that found in 1967. However, in contrast to the colonization pattern in 1967, the Tanytarsini increased very rapidly during this second summer and became a dominant group among the Chironomidae. The species that colonized at the time of refilling were the same as those found during the early part of 1967

whereas the dominating forms during the later part of the summer of 1968 were *T.* (*Cladotanytarsus*) spp., Group A, and *Micropsectra* spp. The marked increase in the average standing crop of these species in 1968 is thought to be a product of the progressive siltation that rendered the habitat more favorable for these forms.

The members of the Chironomini were the most important component of the Chironomidae fauna in 1967, both in the number of individuals and the number of species present in the fauna. *Chironomus attenuatus* grp., *C. nervosus*, *C. abortivus*, and *C. tenuicaudatus* first colonized the reservoir in early May. *Chironomus nervosus* increased in abundance throughout the summer of 1967 (Fig. 6) and then after overwintering in small numbers established a population that was somewhat smaller during the summer of 1968. *Chironomus abortivus* and smaller numbers of *C. tenuicaudatus* increased rapidly throughout the summer of 1967 (Fig. 6) and maintained a combined population averaging

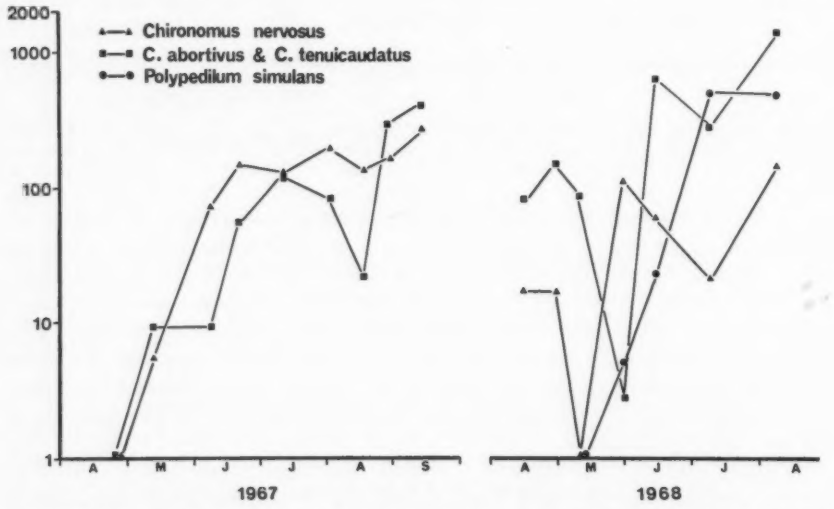

FIG. 6. Population development of *Chironomus nervosus*, *C. abortivus*, *C. tenuicaudatus*, and *Polypedilum simulans* in Laurel Creek Reservoir during 1967 and 1968. Expressed as average number/m^2.

383/m^2 immediately prior to reservoir drainage in the fall of 1967. These species that overwintered in small numbers appeared to emerge in May of 1968. The new generations established in the reservoir increased in abundance until the average population density recorded during the latter part of the study period in 1968 was substantially larger than that found in 1967.

Chironomus attenuatus grp. was particularly well adapted to the conditions prevailing in Laurel Creek Reservoir in the period after it was first filled. Approximately 5 weeks after it was first found in the reservoir this species group had developed an average population density of 10,750/m^2 (Fig. 7)

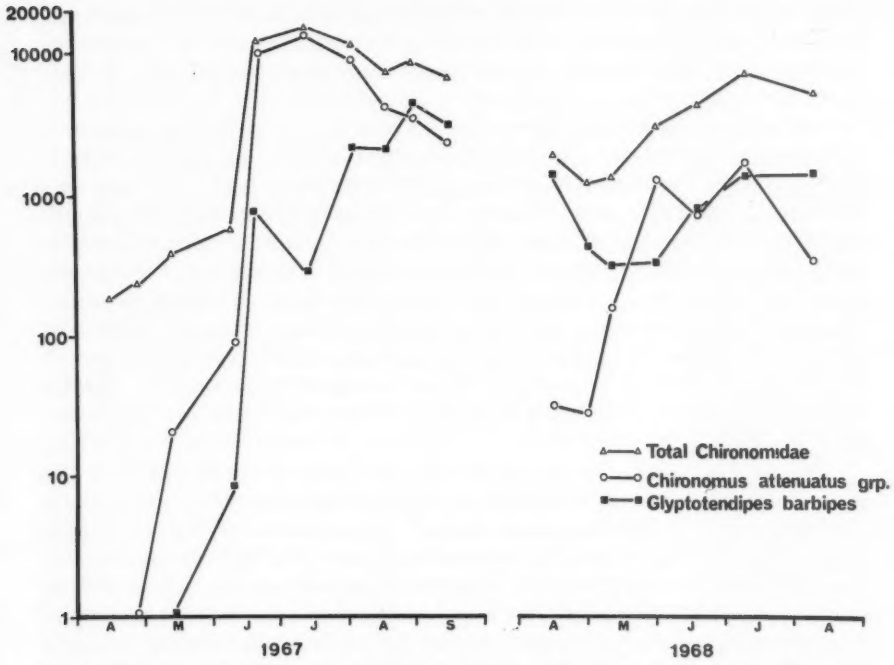

FIG. 7. Population development of the total chironomid fauna, *Chironomus attenuatus* grp., and *Glyptotendipes barbipes* in Laurel Creek Reservoir during 1967 and 1968. Expressed as average number/m².

or approximately 87.3% of the total chironomid fauna of the reservoir at that time. After reaching a peak density of 14,240/m² in early July the population declined steadily to a density of 2350/m² prior to reservoir drainage. Collections of adult chironomids from the region of the reservoir at weekly intervals, plus experimental rearing of larva of *C. attenuatus* group in the laboratory, would suggest that this group passed through several generations in 1967 and that emergence and oviposition were not synchronised but both these phenomena were extended over the entire summer period. Therefore it is postulated that the steady decline in population density of *C. attenuatus* grp. during the latter part of the summer of 1967 was a result of decreased carrying capacity of the habitat rather than larval mortality. This is well substantiated by the results for 1968. After overwintering in large numbers the population of this species group reached a maximum density of only 1800/m² in 1968. All larvae of this group agree closely with the descriptions of *C. attenuatus* (=*decorus*) given by Roback (1957), Johannsen (1937), and Malloch (1915). Numbers of these larvae were reared and the emerging adults were found to belong to the two species, *C. attenuatus* and *C. atrella*, in a ratio of approximately 2:1. Large numbers of larvae collected from the reservoir in

1968 were mounted and carefully compared with larvae from a small pond where rearing experiments had shown only *C. attenuatus* to be present. No anatomical or size differences were found that would suggest the presence of more than one species of larva.

In early June of 1967, or about 1 month after the first appearance of *C. attenuatus* grp. larvae, *Glyptotendipes barbipes* invaded the reservoir (Fig. 7). The population of this species increased rapidly, and from late June until the drainage of the reservoir it was codominant with *C. attenuatus* grp., the two species accounting for more than 80% of the total chironomid fauna on all subsequent sampling dates. This species is the chironomid found best able to survive in the frozen, exposed substrate of the reservoir during the period of winter drainage (Paterson and Fernando, 1969b) and it consequently dominated the chironomid fauna on the first sampling date in 1968. Despite the large population found immediately after the reservoir was refilled *G. barbipes* did not maintain a population that exceeded 1500/m^2 during the second summer of the study.

In addition to the species that dominated the fauna during 1967 and 1968 there were a number of other species of Chironomini found in the reservoir (Table 3). In 1967 *Chironomus brachialis*, *C.* (*Cryptochironomus* sensu Townes, 1945) spp., *Paralauterborniella nigrohalteralis*, and *Stictochironomus* sp. were found sporadically and in small numbers. *Paratendipes* spp. was introduced from the creek at the time of first filling and then was absent, except for small numbers collected in mid-August, during the latter part of the summer. The frequent occurrence, in 1967, of *Polypedilum* spp. and *Phaenopsectra* spp. would perhaps indicate the establishment of a true lentic population. Both genera were found in Laurel Creek but it is not known if the species present were the same as those recorded from the lentic habitat. In 1968 *P. nigrohalteralis*, *Stictochironomus* sp., and *C. brachialis* were not found in the fauna, and *Pseudochironomus fulviventris*, *Chironomus tentans*, and *Polypedilum simulans* were first recorded. *Polypedilum simulans* is of interest as it was absent in 1967 but maintained populations as dense as 504/m^2 in July of 1968 (Fig. 6). The distinctive larva of this species was not found in the creek fauna.

DISCUSSION

The lentic environment established by the closure of a dam across Laurel Creek would be regionally eutrophic because of its southern Ontario location as well as morphometrically eutrophic by virtue of its shallow nature and exposure to the prevailing winds. The physical eutrophic predisposition of the habitat would be further enhanced by the large quantities of organic detritus present on the bed of the reservoir basin prior to impoundment. The potentially eutrophic nature of the developing environment would suggest that a benthic community rich in numbers and species would colonize the habitat.

When the benthic fauna was first examined 2 weeks after the reservoir filled in 1967 the benthos was found to be composed of submerged terrestrial

organisms and a creek-derived assemblage of oligochaetes, nematodes, oribatoid mites, and chironomids. Although the numbers of terrestrial organisms were small the large size of the common species contributed to an appreciable biomass. The creek-derived aquatic species consisted of two groups that could be divided on the basis of their ability to establish permanent populations in the reservoir. The oligochaetes, nematodes, oribatoid mites, *Procladius freemani*, and certain species of Tanytarsini, are representative of a group of facultative species that can thrive in both lotic and lentic environments. The majority of these species showed a population increase within the reservoir and were the major component of the benthic fauna during the early part of the summer of 1967. The other group of organisms derived from the creek fauna consists of species, such as the introduced orthoclads, that are obligate rheophilic organisms and are unable to establish permanent populations within the newly formed lentic habitat. The orthoclads were a major component (47.8%) of the chironomid fauna when the reservoir was first filled but these species then perished or emerged, as only rare specimens of the introduced forms were found after the end of June.

As the populations of submerged terrestrial organisms and obligate rheophilic species were decreasing in May they were replaced by a true limnophilic fauna derived from other lentic environments. As these species, such as *Chironomus attenuatus*, *C. abortivus*, *C. tenuicaudatus*, *C. nervosus*, and *Glyptotendipes barbipes*, are adapted to life in standing waters their populations increased very rapidly and by the end of June they were numerically more abundant than the facultative fauna. The rapid increase in the population densities of most limnophilic species was correlated with a rapid warming of the reservoir water. The pattern of establishment of the benthic fauna of Laurel Creek Reservoir during the first few months after initial filling is very similar to that found for two large reservoirs on the Volga River in Russia by Morduchai-Boltovskoi (1961). However, the warm temperatures on Laurel Creek Reservoir resulted in the sequential pattern of colonization developing much more rapidly than that recorded in the reservoirs on the Volga River by Morduchai-Boltovskoi (1961).

During midsummer of 1967 the benthic fauna of the reservoir was dominated by oligochaetes *C. attenuatus* grp. and *G. barbipes*. As the reservoir substrate was modified by siltation and decomposition of the organic detritus the population of *C. attenuatus* grp. showed a marked decline. *Glyptotendipes barbipes* also displayed population declines from the summer maximum. These decreases may be related to the modifications of the organic substrate. In the period prior to reservoir drainage in 1967, when the population densities of the above mentioned species tended to decline, the abundance of such species as *Chironomus abortivus*, *C. tenuicaudatus*, *Procladius freemani*, and certain members of the Tanytarsini displayed population increases. These changes in the population structures of the various species is thought to result from modifications of the environment rather than being the result of larval mortality. This is substantiated by the rearing of chironomid larvae and the collection

of adults from the shore of the impoundment that suggest that the reproduction of most species was nearly continuous throughout the summer.

The drainage of Laurel Creek Reservoir disrupted the processes of population change within the habitat but, as shown by Paterson and Fernando (1969b), many of the limnophilic species overwintered in the frozen substrate or in two small pools of water remaining in the reservoir basin. The reestablished creek served as a refugium for certain of the facultative species. As a result of the overwintering of many species the pattern of colonization after refilling in 1968 was appreciably different from that observed in 1967. The submerged terrestrial organisms were absent and the fauna consisted of substantial populations of many limnophilic species in addition to facultative and obligate rheophilic forms.

In the summer of 1968 the population densities of the oligochaetes, nematodes and oribatoid mites were not greatly different from those of 1967. These are facultative species that are successful in a wide variety of habitats and thus the modifications of the reservoir environment with time did not appreciably affect this group of organisms. In contrast, the population densities of many of the limnophilic chironomid species changed markedly during the two summers of the study, presumably as a result of the modifications of the habitat. The population densities of *C. attenuatus* grp., *C. nervosus*, and *G. barbipes* found in 1968 were much lower than those recorded in 1967 whereas the changes in the habitat appeared to favor *P. freemani*, *C. abortivus*, *C. tenuicaudatus*, *P. simulans*, and certain species of the Tanytarsini. The changes in the average abundances of many chironomid species caused a marked change in the dominance hierarchy (Table 4). The change from a fauna dominated by *C. attenuatus* grp., and *G barbipes* in 1967 to one in which *C. abortivus*, *C. tenuicaudatus*, and the Tanytarsini are important would be similar to the differences found when comparing the bottom fauna of a eutrophic lake to one that is less eutrophic or perhaps mesotrophic (Deevey, 1941). The changes in the dominance hierarchy of the chironomid fauna of Laurel Creek Reservoir are reminiscent of the pattern recorded in Barrier Reservoir by Nursall (1952).

TABLE 4. The relative importance of various chironomids as a percentage of the total chironomid fauna of Laurel Creek Reservoir during the first two summers of impoundment.

	1967	1968
Tanypodinae	1.8	6.3
Orthocladiinae	0.5	1.1
Chironomus attenuatus grp.	67.7	19.3
C. nervosus	1.7	1.5
C. abortivus and *C. tenuicaudatus*	1.3	10.7
Glyptotendipes barbipes	24.3	26.5
Phaenopsectra spp.	0.5	0.3
Polypedilum simulans	–	5.1
Other Chironomini	0.4	5.0
Tanytarsini	1.8	24.2

Barrier Reservoir is climatically and morphometrically oligotrophic but the preparation of the site resulted in the substrate being covered with large quantities of organic debris. In this habitat the dominant chironomids passed through the sequence *Pentapedilum–Chironomus–Tanytarsus* as the organic detritus was silted over and the habitat became more oligotrophic.

The yearly differences in the average densities of certain of the minor elements of the benthic fauna, such as *Agraylea multipunctata* and *Hyallela azteca*, are associated with the progressive colonization of the reservoir by macrophytes, particularly *Potamogeton foliosus*. The maintenance of a total standing crop of benthic fauna during July and August of 1968 that was approximately equal to that found in the same period of 1967 was largely a result of a substantial increase in the population densities of oligochaetes that served to partially offset the marked population decline of the total chironomid fauna. In this study the benthic fauna has been considered on the basis of numerical abundance. If biomass was considered it would be found that the decrease in the chironomid fauna in 1968 would be more pronounced as the species that came to dominate the fauna were small in relation to the euryoxybiontic forms that dominated in 1967.

It has been suggested that the marginal macroinvertebrate fauna of Laurel Creek Reservoir was essentially complete, with respect to species composition, by the end of the first summer of the reservoir's existence (Paterson and Fernando, 1969a). This also appears to be true with the benthic fauna. Among the Chironomidae, *Chironomus brachialis*, *Paralauterborniella nigrohalteralis*, and *Stictochironomus* sp. were only found in 1967. The restriction of these species to 1967 and the finding of *Pseudochironomus fulviventris*, *Chironomus tentans*, *Anatopynia dyari*, and *Tanypus carinatus* only in 1968 may not be significant as they were all found in very small numbers. *Polypedilum simulans* was absent from the fauna in 1967 but became important during 1968.

ACKNOWLEDGMENTS

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Physical and Chemical Limnology of Two Mountain Lakes in Banff National Park, Alberta[1,2]

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Anderson, R. S. 1970. Physical and chemical limnology of two mountain lakes in Banff National Park, Alberta. J. Fish. Res. Bd. Canada 27: 233–249.

Limnological investigations of an alpine and a lower subalpine lake in Banff National Park from 1966 to 1968 showed that vernal circulation may not extend to the bottom in either lake and that complete autumn circulation may persist for several weeks in both lakes. Winter heat incomes were similar in the lakes, but the summer heat income was much lower for the alpine lake due to heat lost through water renewal. Thermal stratification was more clearly defined in the subalpine lake each year than in the alpine lake. When well-defined chemical stratification occurred, it was short-lived in both lakes. With the exception of phosphate, which was highest in spring and early summer, most changes in chemical composition appeared related to dilution. Very low oxygen concentrations occurred in the bottom water strata of the subalpine lake, but depletion was never severe in the alpine lake. Of the incident visible light 16% penetrated ice and snow cover (1 m) on the alpine lake, but less light reached the water of the lower subalpine lake because of cloudy ice and deeper snow. The trophogenic zone extended to the bottoms of both lakes (about 13 m) for at least part of each year.

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INTRODUCTION

Although many detailed physical and chemical limnological investigations have been conducted in alpine lakes of other countries (e.g., Löffler, 1968; Pechlaner, 1966; Pennak, 1955, 1968; and others), there have been few studies of alpine and subalpine lakes in western Canada, especially in winter. Rawson (1942, 1953) surveyed some large mountain lakes in western Canada, and some unpublished studies of other lakes have been made.

Preliminary to ecological studies on some lakes in Banff National Park, investigations of water temperatures, snow and ice cover, light penetration, and several aspects of chemical limnology in Herbert and Snowflake lakes were conducted in 1966, 1967, and 1968. Herbert Lake was included in a survey study by Rawson (MS, 1939) and in a study of primary production in some mountain lakes by Fabris (MS, 1966), but no previous investigations had been carried out at Snowflake Lake.

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[1]Carried out as part of the Canadian Wildlife Service program of limnological research in the Canadian National Parks.

[2]Some of the Snowflake Lake data was taken from a thesis submitted by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the University of Calgary, Calgary, Alta.

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Snowflake Lake is 50 km north and Herbert Lake is 60 km northwest of the town of Banff, Alberta (inset, Fig. 1). Other lake data are summarized in Table 1.

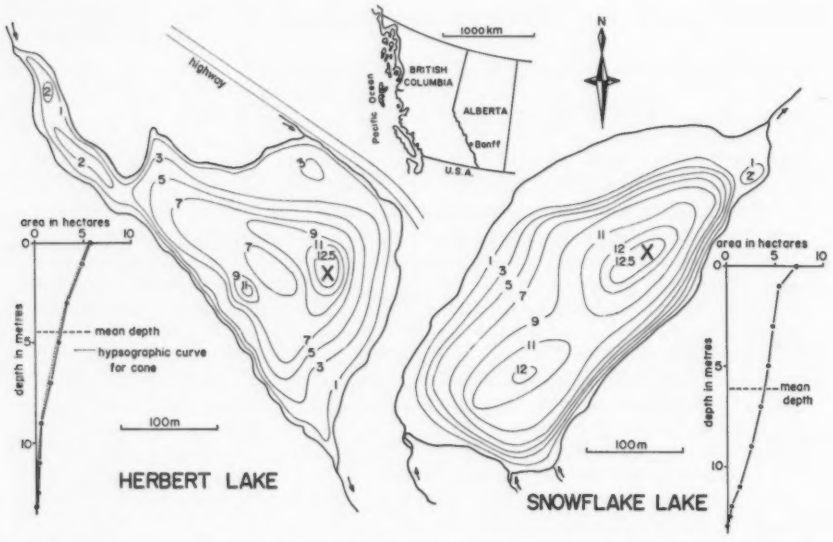

FIG. 1. Bathymetric maps and hypsographic curves for Snowflake and Herbert lakes, Banff National Park. All depths are in meters. The main sampling location at each lake is marked "X."

TABLE 1. Locations, elevations, and morphometric data for Snowflake and Herbert lakes.

	Snowflake Lake	Herbert Lake
Location	51°36′N; 115°50′W	21°28′N; 116°13′W
Grid reference	11U/809167/NH	11U/542010/NH
Elevation (*m*)	2320	1615
Surface area (*ha*)	7.1	5.7
Max depth (*m*)	13.0	13.3
Mean depth (*m*)	6.1	4.5
Approx volume ($\times 10^4 m^3$)	43.6	25.4

Situated in a cirque valley and bordered by a meadow, Snowflake Lake is near treeline in the "alpine zone" (Pechlaner, 1966). There is no higher emergent or benthic vegetation in the lake. Herbert Lake is in the lower "subalpine zone" (Rowe, 1959) and is bordered on three sides by stands of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*). There is a narrow band of emergent vegetation (*Carex* sp.) along part of the shoreline. Scattered clumps of *Chara* sp. and *Potamogeton* sp. occur in the shallow water (Fabris, MS, 1966). Most of the shallow areas of the lake are devoid of higher plant forms.

Although occasional air temperatures of 26 or 27 C were reached in July or August in the vicinity of Snowflake Lake from 1966 to 1968, mean daily air temperatures for the June to September period were in the 5–10 C range. Daily means for the winter period were usually in the −12 to −4 C range with records as low as −38 C in the January–March period (Baptie, MS, 1968). Although records for the cirque area are few and incomplete, it is estimated that the annual precipitation, expressed as the equivalent of rain, ranges between 50 and 70 cm, most of which falls as snow. The alpine zone in this region is subject to winds of up to 80 km hr^{-1} or more, most frequently in autumn and winter.

The forest shelters Herbert Lake from winds, which are usually lower in velocity in the lower subalpine than in the alpine zone. Thirty-five years of weather records for Lake Louise Junction near Herbert Lake indicate that air temperatures fluctuate greatly throughout the year and from year to year (unpublished Department of Transport records). Since 1935, the high and low air temperatures for the November to April period were 23.3 and −52.8 C and the high and low for the May to October period were 34.4 and −27.8 C. The mean daily winter air temperatures are usually in the −8 to +4 C range and the mean daily summer air temperatures are usually in the 10–20 C range. Temperatures well above freezing occur frequently during the winter. Expressed as the equivalent of rain, the total annual precipitation in the Herbert Lake vicinity is usually between 60 and 70 cm, about 60% of which falls as snow.

METHODS

Most samples and measurements were taken at one station at the deepest part of each lake (Fig. 1). Water samples were taken with a Van Dorn-style PVC sampler and stored in polyethylene bottles that were acid-washed and triple-rinsed in demineralized water. Field analyses of alkalinity, hardness, *p*H, and specific conductance were performed within half an hour of sampling whenever possible. Specific conductance was measured with a Dionic Series 3 Conductivity-Meter; *p*H was determined with an E.I.L. Model 308 *p*H Meter; total alkalinity and hardness were determined in the field with chemicals from the Hach Chemical Co., Ames, Iowa. Laboratory water analyses were performed by the Inland Waters Branch, Department of Energy, Mines and Resources of Canada according to standard methods (American Public Health Association, 1965). Dissolved oxygen was measured with a Lakes Instrument Co. Mark II oxygen sensor. Samples of bottom sediments, taken from the deepest parts of both lakes during the winter of 1967, were dried at 100 C for 24 hr and then ashed at 650 C for 2 hr. No corrections were made for the transformation of carbonates and bicarbonates into oxides.

Soundings were made with a Lowrance "Fish-Lo-k-tor" transistorized echo sounder that was checked against a leaded line. Lake volumes were calculated according to the method suggested by Welch (1948). The outlet stream flow from Snowflake Lake was estimated in 1967 on a sandy bottom stretch of uniform dimensions using a floating chip.

Temperatures were determined with a Yellow Springs Instrument Co. Model 425C thermistor thermometer calibrated against a mercury thermometer at each use. The heat incomes for both lakes were calculated according to Hutchinson (1957) and incorporated a 0.9 expansion factor for ice (Pechlaner, 1966). Heat required to melt snow was included for Herbert Lake, but not for Snowflake Lake because little snow remained on the ice at the time of the spring thaw. Nine cm of snow was taken to be equivalent to 1 cm of ice.

No sunshine recorder was available at the time of these investigations. Estimates of hours of daylight for the two lakes were based on field observations and on extrapolations from sunrise–

sunset tables from the Calgary International Airport. Incident light at the lakes was measured with a Sekonic photometer.

Light penetration into the water was measured with a modified underwater photometer and GKA 48-BO1 galvanometer supplied by The International Agency for ^{14}C Determination. The photometer was fitted with a selenium cell that is sensitive only to the short-wave radiation between 350 and 700 mμ (visible light). Filters used with the underwater photometer had the following specifications:

	Schott BG-7	Chance-Pilkington OGr-1	Schott RG-2
Max transmission (mμ)	475	530	660
Range (mμ)	300–600	450–600	590–835

Underwater readings were corrected in the manner outlined by Westlake (1965). In winter, a 15-cm hole was bored in the ice and efforts were made to minimize snow cover disturbance. Before light readings were taken, the ice-hole around the cable was packed with snow, a piece of opaque rubberized fabric was placed around the cable, and snow cover was restored as closely as possible to its undisturbed condition.

PHYSICAL CHARACTERISTICS

Morphometry, Water Renewal, Bottom Sediments

In general the basin of Snowflake Lake is shaped like a flanged bowl whereas that of Herbert Lake approximates an inverted cone (hypsographic curves, Fig. 1). The drainage basin for Snowflake Lake is only 150 ha, which is approximately 21 times the lake area and only about half the comparable figures (mean = 40.5 times) given for 32 Italian alpine and subalpine lakes by Ravera and Tonolli (1956).

The estimated annual water renewal (7.7 times, 1967), most of which occurs between mid-June and mid-August, was similar to the rates determined for the Austrian Finstertaler Seen (large = 2.8 times; small = 11.2 times) by Pechlaner (1966). Snowflake Lake has a volume similar to that of the smaller Austrian lake. Drainage basin area and water renewal rates for Herbert Lake were not determined, but the water renewal rate for this lake is probably in the order of once a year.

The bottom material in Snowflake Lake is mostly fine gravel on the steeper slopes, but the sediment in the deepest areas consists of black mud rather low in organic content. The upper strata of the deepwater sediments in Herbert Lake are medium to dark brown, flocculent, and higher in organic content. The average weight loss for the Snowflake samples was 20.1% of the dry weight; for Herbert samples, 65.4%.

Ice and Snow Cover

Snowflake Lake was usually frozen from about October 10 until late June or early July and Herbert Lake from about the end of October until mid-May (Fig. 2). Snowflake Lake remained blown free of most snow during much of each winter, but snow cover on Herbert persisted throughout the winter. Consequently, the ice layer tended to be somewhat thicker on Snowflake Lake. When ice holes were cut at Snowflake, the water level always

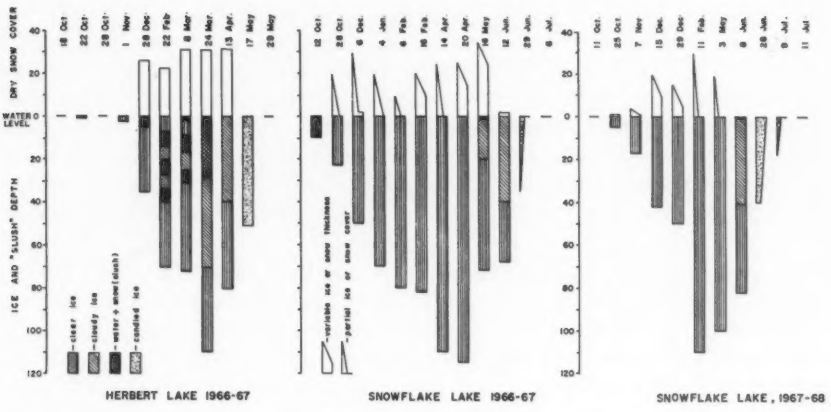

FIG. 2. Ice and snow cover on Herbert and Snowflake lakes. Depths are in centimeters.

remained a few centimeters below the upper ice surface, except in a small area on one side of the lake where deep snow accumulated.

In Herbert Lake, the weight of snow often pushed the ice downward and caused water to well up through cracks into the snow, where the upper layer froze if temperatures were sufficiently low. This resulted in the formation of alternate layers of ice and slush and hence a solid layer of cloudy ice during spells of very cold weather, in sharp contrast to the predominantly clear ice of Snowflake Lake. Because total ice thickness tended to remain constant on Herbert Lake from January to April, the lower surface of the ice layer must have melted off continuously. The lower half of the ice layer was usually fairly clear and rather soft, which suggests that the crystal structure of the ice changed as the temperature increased.

TEMPERATURE

In Snowflake Lake, a thermocline developed in 1967, but was poorly defined in 1966 and 1968 (Fig. 3). The 1967 thermocline was not greatly different from that in Vorderer Finstertaler See in 1961 (Pechlaner, 1966). Under the ice, lower water temperatures occurred in Snowflake Lake at all depths than were found in the Austrian lake, where the water temperature was virtually isothermal at 4 C. The isopleths for Snowflake Lake indicate a rather short period of true isothermy at ice breakup, the slow development of thermal stratification, and a long period of autumn isothermy. Wind velocities during autumn were influential in lengthening the autumn overturn and in producing the low water temperatures that persisted throughout the winter. The lower summer water temperatures in 1966 and 1968 were undoubtedly due to higher water renewal rates, heavier rainfall, and more overcast skies in those years than in 1967.

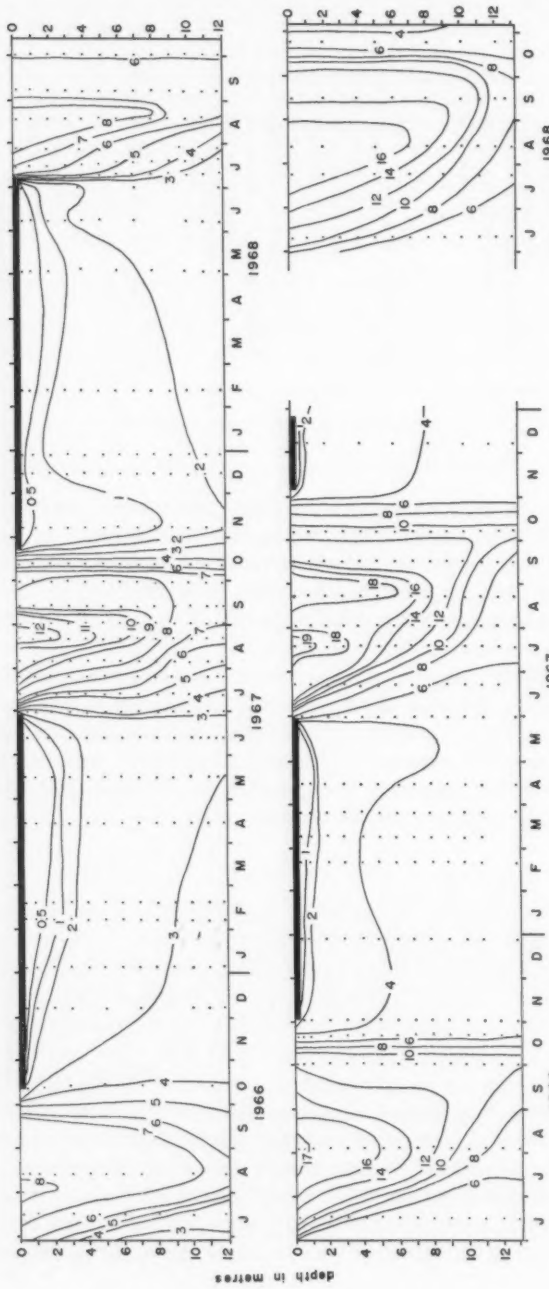

FIG. 3. Temperature (Celsius) isopleths for Snowflake Lake (*upper*) and Herbert Lake (*lower*) during 1966–68.

The higher surface temperatures and marked thermocline in Herbert Lake in 1967 were reflections of the warmer summer that year. Spring isothermy was short-lived, but autumn isothermy persisted about as long as in Snowflake Lake. Temperatures under the ice were higher than those in Snowflake Lake, where autumn winds were colder, more frequent, and higher in velocity. When Herbert Lake was frozen over, slightly higher temperatures at mid-depth than at bottom (usually 0.1–0.2 degrees C higher) suggested the presence of warm springs or limited heating from the bottom sediments between the 5 and 7 m depths.

The cold, wet summer of 1966 partly accounted for a lower summer heat income that year for Snowflake Lake (Table 2). Differences in lake inlet and outlet temperatures during the open-water season in 1967 indicated that a heat loss of up to 9000 cal cm^{-2} occurred through water renewal. The greatest actual heat loss from this source occurred during July, but the greatest difference between inlet and outlet temperatures occurred in September, a feature probably common to most north temperate zone lakes (see Rodgers and Anderson, 1961). Although water renewal rates and inlet–outlet temperatures are not available for Herbert Lake, the summer heat loss due to water renewal would be small compared with Snowflake Lake, where loss due to water renewal could amount to from two to four times the calculated summer heat income.

TABLE 2. Heat budgets for Snowflake Lake, 1966, 1967, and for Herbert Lake, 1967, 1968 (cal cm^{-2}).

	Winter heat income	Summer heat income	Annual heat budget
Snowflake Lake 1966	8,145	2,215	10,360
1967	8,135	4,345	12,480
Herbert Lake 1967			
Not including snow cover	7,577	6,004	13,581
Including snow cover	12,167	–	18,171
Herbert Lake 1968			
Not including snow cover	7,583	5,367	12,950
Including snow cover	10,263	–	15,630

If the heat required to melt the accumulated snow on the lake is included, then the winter heat income for Herbert Lake was much higher than that for Snowflake Lake. This may be due in part to higher air temperature at Herbert Lake and to warm inlet water or heating from the bottom. The higher winter heat income for Herbert Lake in 1967 than in 1968 was required to melt the nearly twice normal snowfall that winter. Incomplete data showed apparently direct relationships between total winter snowfall and ice breakup dates.

Light

For both lakes, the sunrise-to-sunset period was estimated to range from about 6½ hr in December to 15½ hr in June. For Snowflake Lake, the screening effect of the mountains was less in summer than in winter because of the position of the mountains and the elevation angle of the sun. The percentage reduction of sunshine was calculated to be between 8 and 26%, about half the screening effect noted for the Austrian Finstertaler Seen (Pechlaner, 1966).

Midday surface light intensities for Snowflake Lake were usually between 64,000 and 87,000 lux on clear days. A maximum reading of 103,300 lux occurred in the forenoon of June 29, 1967, when the mountains were snow-covered and the sun was near the summer solstice. The next highest intensity was recorded April 20, 1967, a day of almost continuous hazy overcast, which indicates the diffusing effect of light cloud or haze. Similar light intensities were recorded for Herbert Lake at about the same time. A low sun angle together with partial white cloud cover can increase the total radiation on the earth's surface by 40% (Hutchinson, 1957). The shielding effect of heavily overcast skies was apparent on June 29 and November 7, 1967. Cloud cover can reduce the possible radiation by up to 50% in winter and 30% in summer (Rodgers and Anderson, 1961).

The extinction coefficients for Snowflake Lake were estimated from the nomograph given by Sauberer (1962), and fell between 0.32 and 0.47, indicating very little light-absorbing substance in the water and little change in the amount present during the open-water period. The sharp rise in light extinction below 9 m on June 29 (Fig. 4) was also found during July. This was the time of high surface runoff, when cold meltwater from the snow around the lake slid down the sides of the lake basin and created 2 or 3 m of turbid water at the bottom. Although the range of selective filters for the underwater photometer was incomplete at the time of these investigations, some readings were taken at Snowflake Lake. The increased attenuation of light in the different spectral ranges was greatest during the period of high water renewal (July) and blue was absorbed at a proportionately higher rate at this time (Fig. 5).

On Snowflake Lake, over 16% of the incident light penetrated more than 1 m of combined ice and snow cover; on Herbert Lake, up to 13% (Fig. 4).

CHEMICAL CHARACTERISTICS

Low oxygen concentrations in Snowflake Lake were confined to the bottom 2 m (Fig. 6). This and the marked gradient at 6 or 7 m throughout most of the year were indications of limited water circulation under the ice and during July and August. The absence of anaerobic conditions was likely due to the accumulation of only small amounts of organic matter. The increase in dissolved oxygen under the ice in mid-May coincided with spring phytoplankton development (Anderson, MS, 1968). Low oxygen concentrations and the absence of sharp dissolved-oxygen gradients under the ice in Herbert Lake were thought to have been due to more circulation and less photosynthesis

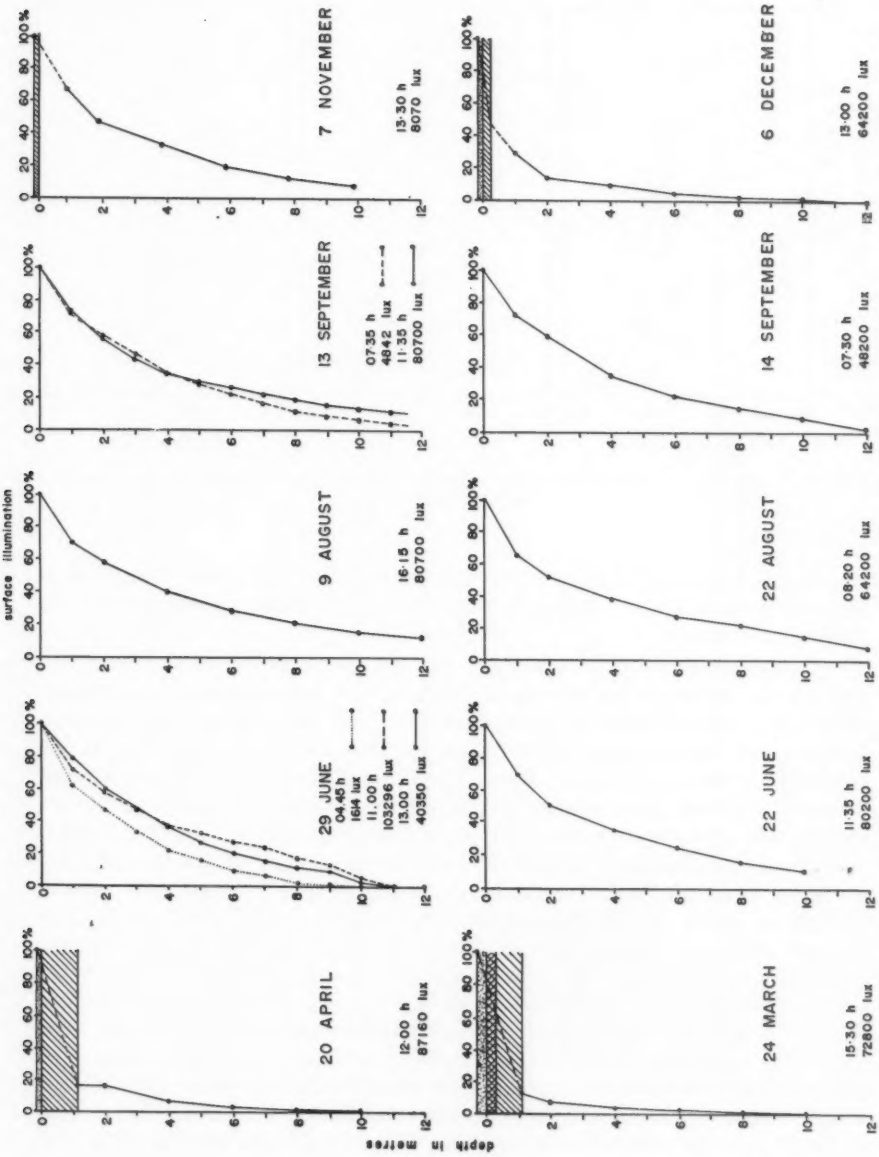

FIG. 4. Penetration of visible light in Snowflake (*upper*) and Herbert (*lower*) lakes as percentages of surface intensity.

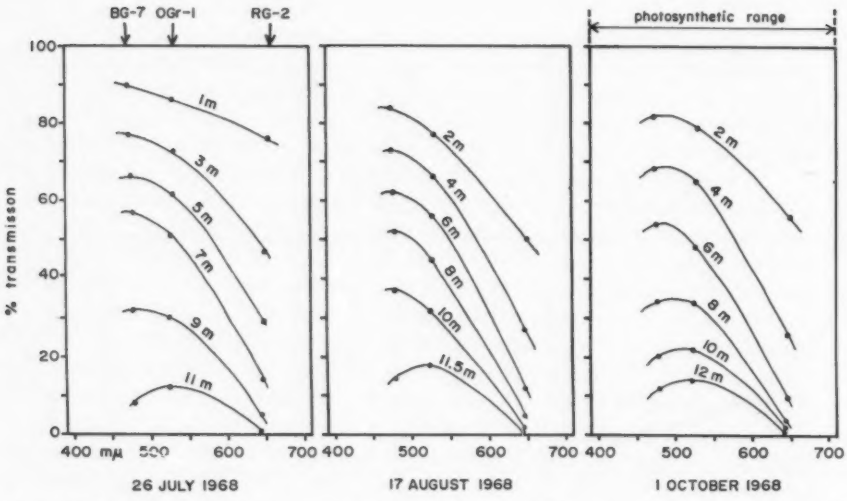

FIG. 5. Penetration of shortwave radiation in Snowflake Lake expressed as percentages of surface intensity.

(Anderson, unpublished data) than occurred in Snowflake Lake. The uniform vertical distribution of oxygen in autumn in both lakes coincided with the prolonged overturn.

Calcium, followed by magnesium, was the dominant cation and bicarbonate was the dominant anion in both lakes (Table 3). However, unlike Snowflake Lake and most lakes in the Banff, Jasper, and Yoho national parks where sulfate is the subdominant anion (Anderson, MS, 1969a, b), chloride was the subdominant anion in Herbert Lake. The order of dominance was not altered by expressing concentrations as milliequivalents per liter, although magnesium, sulfate, and chloride become more important in terms of reacting values.

The ratio of salinity (or sum of constituents as ppm) to the specific conductance as μmhos cm^{-1} at 25 C is between 0.55 and 0.70 for most waters (American Public Health Association, 1965). This ratio for Snowflake and Herbert lakes fell consistently between 0.47 and 0.56 with an approximate mean value of 0.53. Specific conductance and sum of constituents had similar seasonal patterns and reached lowest surface values in June, July, and August (Fig. 7, *c*, *d*, *g*, *h*), when water renewal and primary production rates were highest in Snowflake Lake. Concentration gradients were more striking in Herbert than in Snowflake Lake and were probably related to the more clearly defined thermocline and the lower summer circulation in Herbert Lake. The probability of incomplete overturn in both lakes in spring is suggested by the isopleths in Fig. 7, although vernal circulation may reach a greater depth in Snowflake than in Herbert Lake.

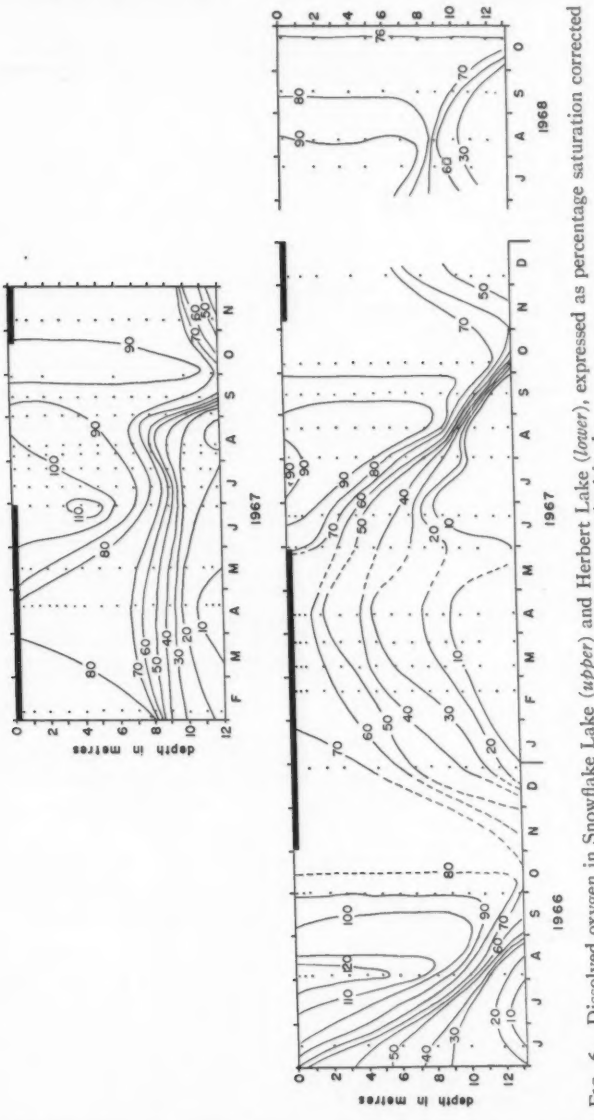

FIG. 6. Dissolved oxygen in Snowflake Lake (*upper*) and Herbert Lake (*lower*), expressed as percentage saturation corrected for temperature and altitude.

Two *p*H maxima in the surface waters of both lakes were found in 1967 (Fig. 7, *b*, *f*). The first occurred under the ice after the time of maximum ice thickness, and the second just after the period of highest water renewal.

In both lakes, the seasonal variations in the concentrations of sodium, potassium, magnesium, calcium, bicarbonate, sulfate, chloride, and silica

TABLE 3. Concentrations (in ppm) of some anions and cations from near surface and near bottom in Snowflake and Herbert lakes during spring and summer, 1967.

	Snowflake Lake				Herbert Lake			
	Apr. 14		Aug. 21		Mar. 8		Aug. 22	
Constituent	2 m	8 m	0.5 m	8 m	2 m	8 m	0.5 m	8 m
Hardness (*total as $CaCO_3$*)	127	122	89.2	92.7	168	169	161	178
Sum constituents	138	135	96.5	105	173	173	163	182
Calcium	32.5	31.7	24.9	21.6	41.0	41.3	40.0	42.0
Magnesium	11.1	10.4	6.6	9.6	15.9	16.0	14.8	17.7
Iron (dissolved)	0.02	0.02	0.01	0.01	<0.005	<0.005	0.01	0.01
Aluminum	0.17	0.10	0.10	0.04	0.01	<0.01	0.12	0.08
Manganese (total)	0.02	0.03	0.010	0.035	0.01	<0.01	0.010	0.020
Copper	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.007	0.006
Zinc	0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	0.005
Sodium	4.7	4.8	1.8	4.5	2.5	2.0	1.8	1.9
Potassium	0.6	0.5	0.2	0.3	0.7	0.7	0.6	0.6
Ammonia	<0.1	<0.1	0.1	0.1	–	–	0.1	0.1
Bicarbonate	140	138	103	117	189	190	179	205
Sulfate	16.6	16.1	10.0	8.3	4.4	4.1	4.8	4.0
Chloride	0.5	0.4	0.1	0.2	8.5	8.4	6.5	8.3
Fluoride	0.05	0.04	0.04	0.04	0.18	0.08	0.08	0.07
Phosphate (diss.)	0.01	0.01	0.01	<0.01	0.02	–	0.02	0.01
Nitrate	0.10	0.22	<0.1	0.1	0.4	0.5	<0.1	<0.1
Silica	2.7	2.9	2.3	2.5	6.8	6.8	6.5	6.4

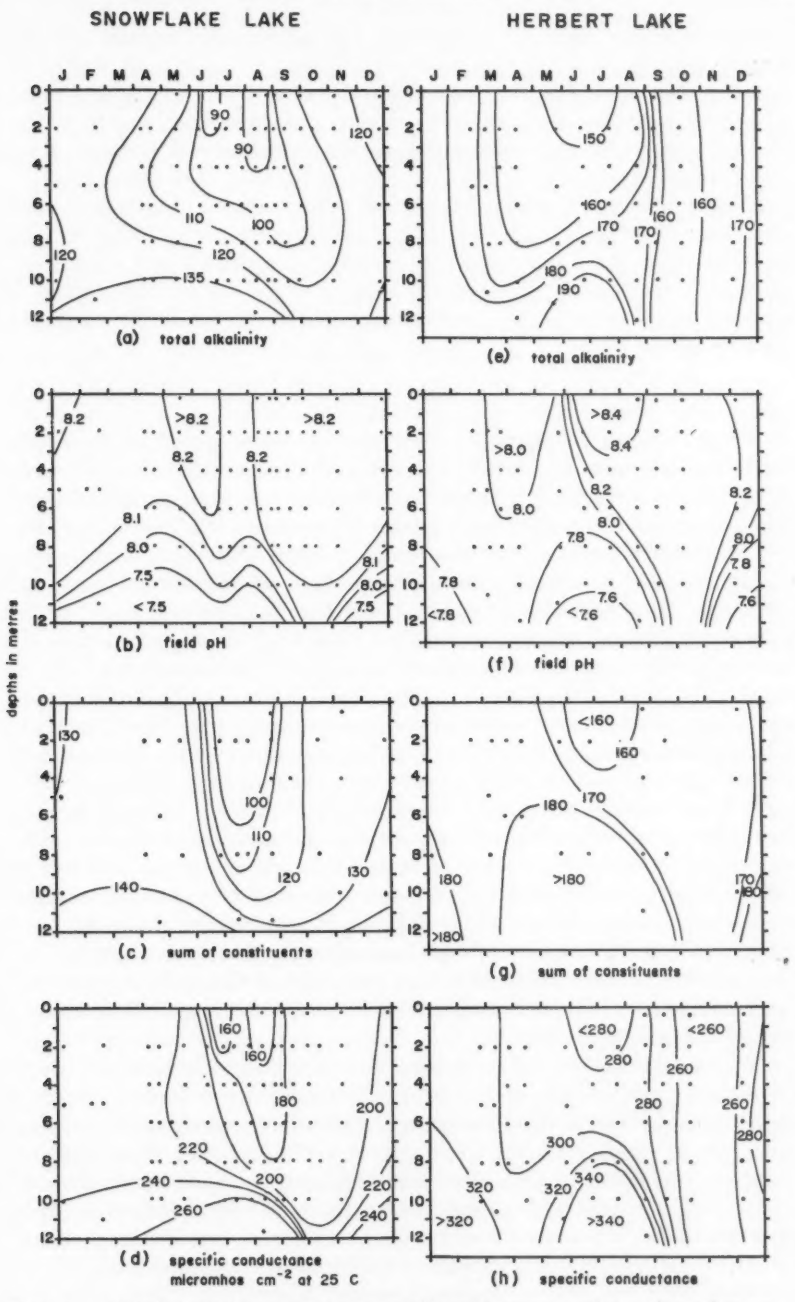
SNOWFLAKE LAKE
HERBERT LAKE
J F M A M J J A S O N D
depths in metres
(a) total alkalinity
(e) total alkalinity
(b) field pH
(f) field pH
(c) sum of constituents
(g) sum of constituents
(d) specific conductance micromhos cm⁻² at 25 C
(h) specific conductance

FIG. 7. Isopleths for pH, total alkalinity, specific conductance, and sum of constituents for Snowflake and Herbert lakes, 1967.

tended to follow closely the patterns for specific conductance and sum of constituents and to reflect the combined influence of flushing and photosynthetic activity. No clear pattern was exhibited by dissolved iron and manganese, but small increases were apparent during and immediately after the period of late winter and early spring stagnation, which would produce a weakly reducing environment in and near the sediments (Gorham, 1958).

No satisfactory methods for lakeside determination of nitrate or dissolved phosphate were available at the time of investigation, but laboratory analyses indicated that the total dissolved phosphate concentrations were very low (Table 3). Exceptions occurred at the time of highest water renewal, when concentrations occasionally doubled or tripled. Nitrate rarely exceeded 0.1 ppm in either lake.

DISCUSSION

With respect to physical and chemical limnology, Snowflake Lake is fairly typical of the alpine and upper subalpine lakes (other than glacial lakes) in the National Parks of Canada, and Herbert Lake is representative of the small, mesotrophic lower subalpine lakes (Anderson, MS, 1968, MS, 1969a, b).

Incomplete vernal circulation in both lakes (Fig. 6, 7) may have been due to low wind velocities, and in Herbert Lake to uniform 4 C temperatures under the ice at the time of ice breakup (Fig. 3). The lack of separation of winter and summer stagnation periods in Herbert Lake (Fig. 6) was probably due to incomplete spring overturn. Rawson (MS, 1939) stated that "adequate" circulation occurred in Herbert Lake, that oxygen saturation near bottom was not lower than 80%, and that stagnation was unlikely to occur. Measurements in the present study indicated that saturation below 50% is likely to occur in the lake in both summer and winter. Saturation levels below 5% were found in the bottom 2 m at both times in 1967 (Fig. 6). Circulation in the lake may be lower now than it once was and the processes of eutrophication, natural or artificial or both, may have accelerated somewhat in the past 30 years. Oxygen supersaturation did not commonly occur in the lakes, but near-saturation levels were common. The infrequent occurrence of supersaturation may have been due to the low photosynthetic production measured in the lakes (Anderson, MS, 1968, and other unpublished data). It is unlikely that oxygen saturation figures for alpine lakes are of value as indicators of production (Pechlaner, 1966).

It is likely that the low concentrations of dissolved iron in the two lakes was due to a combination of factors: to the small amount of dissolved organic matter present that could function as a chelator; to the iron-poor geological formations in which the lakes occur (Baird, 1967; McLaren, 1955); and to the infrequent occurrence of anaerobic conditions in the lakes. Seasonal *p*H maxima (Fig. 7, *b*, *f*) coincided with periods of low circulation and with peaks of photosynthetic activity (see Arnemo, 1964).

Although visible light constitutes only about half the total incoming irradiance as measured by a pyrheliometer (Strickland, 1958), it has been

pointed out (Smith, 1968) that the total energy between 350 and 700 mμ characterizes the photosynthetically useful radiant energy in natural waters. Although the photometer output at each depth in this study was indicative of the total light energy at that depth, the relationship is complex because different wavelengths are selectively filtered out by the water and because the selenium cell varies in its sensitivity to wavelengths between 350 and 700 mμ (Westlake, 1965). The percentage of incident visible light that penetrated the ice and snow cover in Herbert and Snowflake lakes was much greater than that reported for some other lakes in winter (Chandler, 1942; Halsey, 1968; Pechlaner, 1964; Pennak, 1968; Rodhe, 1962; Wright, 1964). In Snowflake Lake, this was undoubtedly due to the high transparency of the ice, little snow or slush on the ice, and the high albido.

The depth at which light intensity is 1% of surface intensity is suggested as the lower limit of the trophogenic zone (Steemann-Nielsen and Aabye Jensen, cited by Arnemo and Nauwerck, 1965). During much of the year, this zone extended to the bottom of Snowflake Lake, and to the bottom of Herbert during some of the open-water season.

The winter heat income for Snowflake Lake (Table 2) was approximately the same as the 7830–8920 cal cm^{-2} calculated for Vorderer Finstertaler See by Pechlaner (1966). Although the ice was thicker and the water colder at Snowflake Lake, the Austrian Lake has a greater mean depth, which probably contributed to the similar heat income. The summer heat income figures for Snowflake Lake were much lower than Pechlaner's figures of 5690–6740 cal cm^{-2} for Vorderer Finstertaler See. This was undoubtedly due to the higher (nearly four times) water renewal rate for Snowflake Lake. The heat loss due to water renewal will be proportionately greater in small lakes having relatively high water renewal rates, and the calculation of summer heat income without considering this source of loss produces an inaccurate figure.

The summer heat income figures for Herbert Lake (Table 2) were much higher than those for Snowflake Lake but slightly lower than those for Vorderer Finstertaler See. The Herbert Lake summer value compared closely with the value of 5650 cal cm^{-2} for Amethyst Lake (Rawson, 1953), and the annual budget for Herbert Lake compares closely with the annual budget of 15,300 cal cm^{-2} determined for Lake Erken in Sweden (Nauwerck, 1963).

There is little positive evidence so far for either lake that enough heat exchange from the bottom sediments occurs to produce circulation as was reported for Tub Lake by Likens and Ragotzkie (1965, 1966). However, the gradual rise of the 2 C isotherm in Snowflake Lake (Fig. 3) in the winter of 1967–68 suggests that heat transfer to bottom waters from the sediments may occur some years.

Thermoclines are "not regularly developed in temperate alpine lakes" (Thomasson, 1956), a feature that is undoubtedly related to the short open-water season and to the prevalence of high velocity winds and low air temperatures at high altitudes. The weak summer thermal stratification in Snowflake Lake and the small zone of low oxygen saturation indicated that less

than 10% of the lake volume was isolated by stratification (Fig. 3, 6, summer 1967). If this is the case, then the actual and estimated water renewal rates were nearly equal.

ACKNOWLEDGMENTS

During the study, valued help and advice were freely given to me by my graduate committee, Drs P. K. Anderson, J. B. Cragg, and R. C. B. Hartland-Rowe. The cooperation of Mr J.-P. Cuerrier and other Canadian Wildlife Service personnel and National and Historic Parks Branch personnel has been greatly appreciated. Thanks are also due to Mr S. W. Reeder, Inland Waters Branch, for useful discussions concerning water chemistry, and to the University of Calgary for laboratory facilities.

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Halosphaera viridis: Fatty Acid Composition and Taxonomical Relationships

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Ackman, R. G., R. F. Addison, S. N. Hooper, and A. Prakash. 1970. *Halosphaera viridis*: fatty acid composition and taxonomical relationships. J. Fish. Res. Bd. Canada 27: 251–255.

The total fatty acids of a natural bloom of *Halosphaera viridis* have been determined. A readily extractable lipid fraction, mostly triglyceride, showed additional monoethylenic fatty acid isomers not obvious in the total lipid. Comparisons are made on the usefulness of this data in distinguishing Chlorophyceae and Prasinophyceae within the Chlorophyta.

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INTRODUCTION

Considerable biochemical evidence has been accumulating to support the separation of certain unicellular algae of the Chlorophyta, formerly classed as Chlorophyceae, into a new class, the Prasinophyceae (Parke and Manton, 1965; Craigie et al., 1966, 1967; Ackman et al., 1968).

The genus *Halosphaera* is of particular interest because of the nonmotile pelagic cyst stage, which is large (reportedly up to 1800 μ diam) by unicellular marine algal standards and succeeds smaller motile stages with flagella (Manton et al., 1963; Parke and den Hartog-Adams, 1965). This recent work of Parke and colleagues has led to *Halosphaera* (considered as three species) being classed with the Prasinophyceae.

Virtually all fatty acid analyses reported for phytoplankters are from cultures (reviewed by Ackman et al., 1968, and Cheucas and Riley, 1969), and few are from natural blooms. Klenk and Eberhagen (1962) reported the fatty acid composition of a *Biddulphia sinensis* bloom, and Patton et al. (1966) the fatty acid composition of a bloom of *Gonyaulax polyedra*. An unusual opportunity to investigate the fatty acids of *Halosphaera* as indicators of taxonomic position occurred when material from a natural bloom was

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collected in Newfoundland waters in early 1969, and the results are reported herein.

The bloom of green algae occurred in the vicinity of Southern Harbour, Placentia Bay, Nfld., in mid-May, 1969. It was suspected that this bloom could have been associated with an unusual enrichment of the water in nitrogens, phosphates, and other chemicals due to the discharge of industrial waste from a phosphate reduction plant commencing operations in late 1968 in Long Harbour, Nfld. Samples were collected by plankton net on May 15, 1969, drained and frozen immediately, and kept at −40 C or at dry ice temperature for 2 months prior to examination.

EXPERIMENTAL

Microscopic examination of part of the frozen algae indicated that the bloom was virtually monospecific in respect to *Halosphaera*, and the sample examined did not contain any apparent zooplankton. The diameters of the cells were remarkably uniform at approximately 350 μ.

Lipids or fatty acids, or both, were recovered from frozen blocks (1–5 g) of algae by treatment under nitrogen with freshly distilled solvents as follows: (*a*) Rapid extraction by intermittent shaking in the monophasic solvent system of Bligh and Dyer (1959), omitting mechanical agitation, with filtration after 10 min, and subsequent transesterification (MeOH–BF_3) of the recovered lipid. (*b*) Saponification for 30 min in refluxing EtOH–H_2O–KOH, with recovery of fatty acids from the acidified solution with diethyl ether, followed by MeOH–BF_3 esterification. (*c*) Extraction as in (*a*), but solvent exposure prolonged to 3–4 hr of extraction. Transesterification was employed on recovered lipids. (*d*) Saponification of the green extracted residue from (*a*), with further processing as in (*b*).

Analytical thin-layer chromatography (TLC) of lipids was carried out with Eastman Kodak Chromagram sheets, and preparative separations of methyl esters on Supelcosil ($AgNO_3$-impregnated silica gel) coated glass plates.

Gas–liquid chromatography (GLC) was carried out on a Perkin–Elmer model 900 on columns 50 m (150 ft) in length and 0.25 mm (0.01 inch) internal diameter. Coatings were butanediol succinate (BDS) or Apiezon-L (ApL). Presentation of data to two decimal places is solely to show minor components and there is no implication of this order of accuracy.

RESULTS

Lipid yields were examined only for experiment (*a*) where a recovery of part only of the lipid was 0.2 mg/g cells (for comparative data see Ackman et al., 1968). The fatty acid compositions (fatty acids only) listed in Table 1, column A, are based on identifications of definite fatty acid components from experiment (*b*) examined on both polar and nonpolar GLC phases, the GLC analyses being correlated with behaviour on $AgNO_3$–silica-gel TLC. A number of other components appeared in quantity sufficient to cause concern, as on superficial examination several coincided with possible fatty acid components on the polar (BDS) columns used for basic GLC work. These have been identified by equivalent chain length (ECL) value and are not esters of acids as indicated by their behaviour on TLC. They are quantitated (column D) with reference to total GLC response. Particular note should be taken of components with ECL 17.97 and 19.15 (respectively corresponding closely to 18:0 and 18:3ω6) in the BDS analysis. Most of these materials were apparent only in the esters derived from acids recovered after direct saponification

TABLE 1. Total fatty acids (weight percent) from natural *Halosphaera* (column A), prominent fatty acids from a largely triglyceride fraction from *Halosphaera* (column B), and total fatty acids[a] from a culture of *Tetraselmis* (*Platymonas*) sp. (column C). Unknown materials from major unknowns or artifacts, or both, from *Halosphaera* analysis listed as GLC recorder area response in column D.

	A	B	C	D
	Halosphaera bloom			
Fatty acid	Total	Fraction	Cultured *Tetraselmis*	GLC unknowns
12:0	0.12	–	0.1	–
14:0	4.95	5.74	0.8	–
ECL 14.20	–	–	–	0.65
15:0	0.02	–	0.1	–
ECL 15.32	–	–	–	0.06
16:0	17.63	19.15	19.7	–
16:1ω9	1.09	1.59	1.0	–
16:1ω7	0.16	3.19	1.9	–
16:2ω9?	0.13	–	–	–
17:0	0.02	–	0.2	–
ECL 17.31	–	–	–	1.54
16:3ω3?	0.07	–	0.2	–
16:4ω3	3.42	2.20	14.7	–
ECL 17.97	–	} 2.23	–	0.98
18:0	0.07	}	0.3	–
18:1ω9	44.75	47.23	8.8	–
18:1ω7	–	2.55	3.0	–
18:2ω9?	0.71	–	–	–
18:2ω6	7.38	4.47	3.3	–
18:3ω6	0.91	0.64	0.6	–
ECL 19.15	–	–	3.3?	3.92
ECL 19.32	–	–	–	2.45
18:3ω3	7.28	5.11	18.9	–
18:4ω3	6.55	4.15	7.5	–
20:0	0.07	–	–	–
20:1ω11	0.02	0.77	–	–
20:1ω9	Trace	0.96	1.6	–
ECL 20.32	–	–	–	1.63
20:4ω6	Absent?	–	1.3	–
20:4ω3	0.39	–	Absent?	–
20:5ω3	Trace	–	7.5	–
22:5ω3	0.43	–	–	–
22:6ω3	3.83	–	1.4?	–

[a]96% of GLC area included but several minor fatty acids omitted.

of the algae. One series of unknowns with fractional chain length (FCL) values of 0.31–0.32 suggests a homologous series of compounds that may include other members masked by 16:1ω9 and 18:1ω9 (ECL values of these are 16.25 and 18.21 respectively).

The absence of animal type fatty acids, such as the *iso* and *anteiso* 15:0 and 17:0 (Ackman and Sipos, 1965), is quite definitely a confirmation of a high degree of purity as a phytoplankter sample.

In column B of Table 1 is given the composition of the major fatty acids from experiment (*a*). Most interestingly, this sample contained additional 16:1ω7 and also 18:1ω7 not observed in the other results, and components tentatively identified as 20:1ω11 and 20:1ω9 present but not obvious in the larger preparation of (*b*). These unexpected monoenes in the esters from

sample (*a*) were verified as to type of acid by the silver nitrate TLC procedure. A qualitative TLC examination of the extracted lipids of (*a*) indicated that the lipid was predominantly triglyceride, accompanied by some free fatty acid and spots with R_f values similar to sterols, mono- and di-glycerides, as well as a polar residue at the origin. It is believed that extract (*a*) represents an especially readily extractable triglyceride lipid, and therefore the fatty acid composition differs in some detail from the acids recovered from more complete digestion or extraction. In the case of experiment (*a*) the residue was observed to contain intact cell walls (possibly pectin or similar material) with appreciable residual colour visible in a liquid phase inside the translucent spheres. The esters from experiments (*c*) and (*d*) were similar in composition to those from (*b*), except for variations in the nonfatty acid materials.

Most *Halosphaera* fatty acids occur widely in algae and have a general strong resemblance to fatty acids of the *Tetraselmis* (*Platymonas*) sp. culture included in Table 1, column C. The prominence of 18:1ω9 possibly reflects involvement in a depot fat role at this mature stage of the *Halosphaera* life cycle. The absence of obvious 20:5ω3 is perhaps the most striking difference between the two species but may be based on the same reasoning, as 22:6ω3 is more pronounced in the *Halosphaera*. On comparing fatty acids with the Chlorophyceae as exemplified by *Dunaliella tertiolecta* and the Prasinophyceae, by *Tetraselmis* sp. (Ackman et al., 1968), the absolute higher level of 18:1ω9, as well as its excess over 18:1ω7, and the lesser proportion of 18:3ω3 but substantial inclusion of 18:4ω3 and of C_{22} polyenes (none in the Halifax Chlorophyceae) are perhaps the important points where these fatty acids from a natural bloom of *Halosphaera* more closely resemble those from the Prasinophyceae cultures.

The other data available for comparison has been published by Cheucas and Riley (1969). This includes fatty acids[1] from cultured *D. tertiolecta* (compare Ackman et al., 1968), *D. primolecta*, two *Chlamydomonas* sp. (all Chlorophyceae), and from *Heteromastix rotunda* (Prasinophyceae, Pyraminonadeles, Nephroselmidaceae). Curiously this data places 20:5ω3 in both the Dunallielaceae, and in *H. rotunda*, but not in the Chlamydomonadaceae. Apparently this acid is not a useful means of distinguishing the Prasinophyceae from the Chlorophyceae. On a qualitative and even semi-qualitative general basis (excluding 20:5ω3), the individual fatty acid percentages for the more obvious components are in moderate agreement in assigning all six algae to the Chlorophyta. However, in some respects it is difficult to find quantitative differences between fatty acids of the two orders greater than the corresponding differences between the two *D. tertiolecta* analyses from different laboratories.

It is often argued that in such comparisons it is preferable to have identical growth conditions, but artificial cultures may in fact be unduly restrictive to natural growth of some unusual species. The environmental conditions and

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[1]Note that the 22:5 (7,10,13,16,19) tentatively listed by these authors is 22:6ω3(4,7,10,-14,16,19).

trace nutrients available for the *Halosphaera* bloom in Newfoundland waters presumably were those commonly available for restricted growth of this organism in its normal habitat. In the larger view, any bloom must result from an unbalance in various factors that normally restrict growth and reproduction of a pre-existing parent stock. There is no reason to consider that the analytical data from this particular natural sample would be more atypical for this species than data obtained from artificial cultures in which growth factors may be unwittingly limited, and which are usually held to a mature stage to provide maximum yields.

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Laboratory and Field Tests of Modified Sphyrion Tags on Lobsters (*Homarus americanus*)

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Mortalities of lobsters held in the laboratory and tagged with sphyrion tags inserted dorsally between the carapace and abdomen (subcarapace tag) and between the second and third abdominal segments (abdomen tag) did not differ significantly. Premoult tag loss was negligible. The subcarapace tag was retained significantly better through moulting.

Returns of subcarapace-tagged and abdomen-tagged lobsters released on the fishing grounds in June, 11 weeks before the August 10 opening of the fishing season, were lower than returns of carapace-tagged lobsters released in early August. More subcarapace-tagged than abdomen-tagged lobsters were returned, and a higher proportion had moulted. Growth was comparable to that reported in the literature. Few lobsters were recaptured during the following fishing season, 14–16 months after liberation, and none subsequently. Returns from lobsters less than 63.5 mm carapace length (sublegal) when tagged were extremely low. Poor survival may be related in part to time of release. Some injury to internal organs may occur with both tags. The subcarapace tag is clearly superior but may be of limited value in the field for lobsters below 63.5 mm carapace length.

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INTRODUCTION

IN COMMON WITH OTHER CRUSTACEA, the lobster presents a problem in that any tag affixed to the shell is lost when the animal moults. Various methods of identification, including marking with holes through sections of the tail fan, have been reviewed by Costello (1963) and Simpson (1963). Other examples are the internal metal "U" tags (Ropes, 1962), toggle tags (Gundersen, 1962, and personal communication), various dart tags (Floy Tag & Mfg. Inc.), staining methods (Costello, 1963), and branding (Abrahamsson, 1965). Scarratt and Elson (1965) described the so-called "sphyrion" tag and gave results of some preliminary experiments.

The original sphyrion tag has been modified by replacing the flat vinyl or polyethylene label with extruded vinyl "spaghetti" threaded over the filament and fused to it in a flame (Fig. 1). There is now less tendency for the label to become hidden in the thoraco-abdominal fold, or for the filament to tangle with the walking legs. Moreover the firm joint between filament and label aids removal of the needle following tag insertion.

There are two types of site for anchoring a tag of this nature: (1) through the thoraco-abdominal membrane, where the shell separates at moulting; and (2) in other areas where the tag must be drawn through the membrane when the shell is shed. Each site requires its own kind of tag. The thoraco-abdominal or subcarapace tag has a long flexible filament to minimize irritation

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at the tag site, with the label remaining outside the shell entirely, whereas the "through the shell" tag has a long label, with the proximal end embedded in the muscle and the remainder, hopefully, smooth enough to pass completely between segments of the old shell at moulting time. The short filament merely joins anchor and label together. This paper reports laboratory and field trials of these two types of tags.

MATERIALS AND METHODS

The two tag types were: (*a*) subcarapace tag with filament and label each 25 mm long, inserted through the thoraco-abdominal membrane; (*b*) abdomen tag with filament 3 mm and label 47 mm long inserted between abdominal segments 2 and 3.

Insertion of the tags was accomplished in the following manner.

Subcarapace tags — One of the ends of the anchor was inserted into the hollow tip of a #20 hypodermic needle and opened slightly to reduce the likelihood of it catching. The lobster was held by the carapace and the abdomen gently pulled downwards to expose the thoraco-abdominal membrane. The needle and tag were then thrust through the membrane to the right of the midline in an obliquely downward, forward, and lateral direction until the tip of the needle touched the inner side of the gill arch and the anchor was located near the insertion of the abdominal extensor muscle. The tag label was held firm against the lobster's back with an index finger while the needle was withdrawn. The operation can be performed by one person but is simpler and more rapid with two.

Abdomen tags — The tag and lobster were picked up in the same manner and the second abdominal intersegmental membrane exposed by flexing the abdomen. The needle was inserted downwards and forwards into the main abdominal flexor muscle to the right of the dorsal midline.

For the laboratory trial, lobsters, approximately 450 g (1 lb), were assigned to individual compartments in plastic trays provided with running sea water at 15 ± 1 C. Earlier experiments had shown that high tag loss occurred unless lobsters were isolated from one another. Lobsters were introduced to the tanks and allowed to acclimate for 14 days before the tags were applied on March 8, 1966. Carapace-tagged animals (Wilder, 1954) were used as controls. The lobsters were fed beef liver, squid, or haddock at the rate of 1% of body weight per day and were examined daily. The experiment ran for 266 days and was terminated on November 20, 1966.

Miminegash, P.E.I., where there has been a continuing study of the lobster fishery by FRB personnel (Paloheimo, 1963; Scarratt, 1964; Wilder, 1965), was selected for the field trial. The lobster fishing season here is from August 10 to October 10 each year. Lobsters obtained from the spring fishery on the north side of the island and released off Miminegash will have opportunity to moult and grow before fishing starts.

Between May 31 and June 2, 1966, approximately 1000 lobsters caught by spring-season fishermen at nearby Tignish, P.E.I., were tagged, half with subcarapace tags, and half with abdomen tags. Sex, carapace length, and tag number of each lobster were recorded. Sizes ranged from 54 to 85 mm carapace length and weights from 130 to 600 g; 300 lobsters were below the legal size limit of 63.5 mm (2½ inches) carapace length. Usually 50 of one tag type were applied, followed by 50 of the other to reduce bias arising from lobsters being tagged at different times prior to release. Lobsters were held in floating crates prior to tagging and were returned to the water in crates within a few minutes of the tag being applied. The crated lobsters were taken to Miminegash by truck each afternoon and released individually in 4–8 fath of water, 1–2 miles off Miminegash Harbour.

After the opening of the lobster season on August 10, fishermen were paid $1.00 for the return of any tagged lobster with details of where and when it was caught. Legal-sized animals had the tag removed and were returned to the fisherman after examination and measuring. Sub-legal animals were returned to the sea with tag attached. A reward of 50 cents was paid for a detached tag.

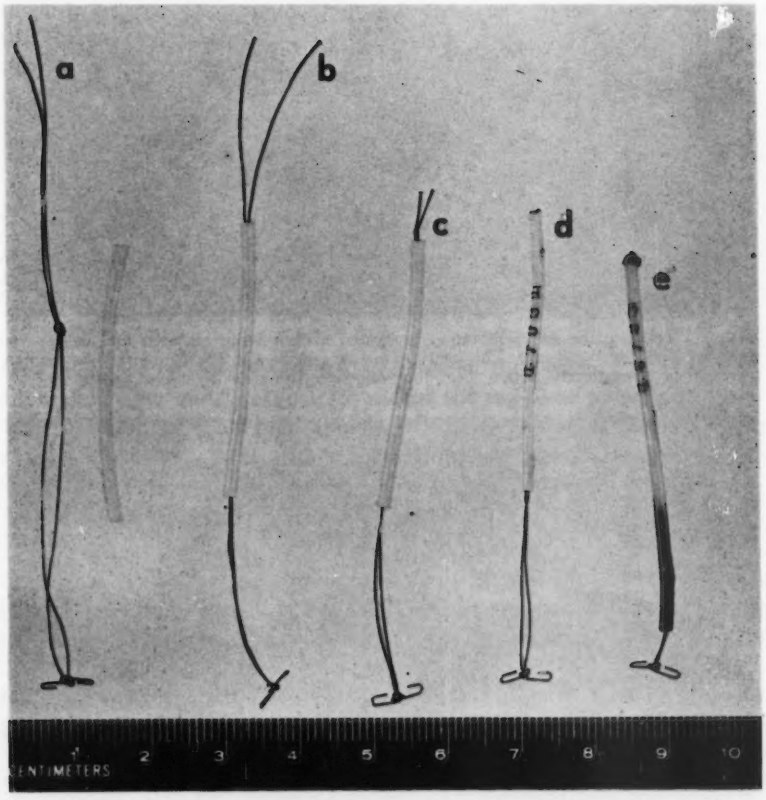

FIG. 1. Two versions of the "sphyrion" tag used in laboratory and field experiments: *a–d* show construction of the subcarapace tag; *e* is the short filament, abdomen tag.

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FIG. 2. Subcarapace sphyrion tag — encysted anchor lying between and below the extensor and flexor muscles. The heart is visible behind the tag filament. The anchor is immediately above, but does not penetrate the digestive gland.

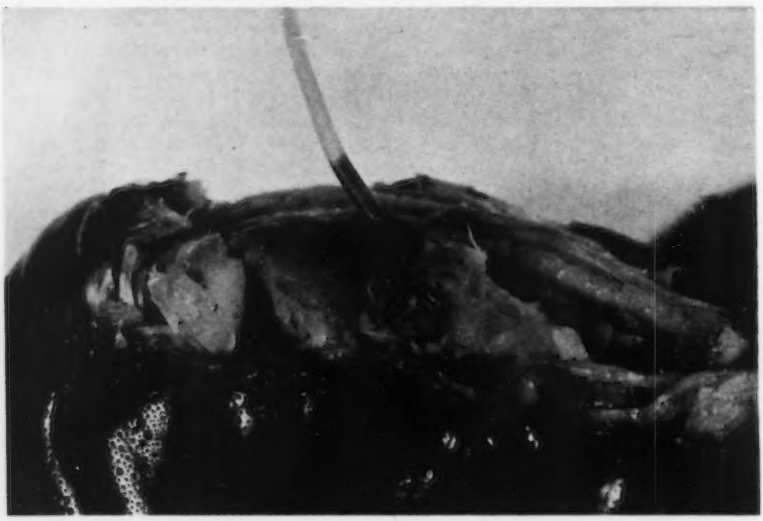

FIG. 3. Abdomen-insertion tag penetrating the abdominal flexor muscle. The extensor has been cut away and the tag anchor exposed. Note the discolouration of the musculature in the vicinity of the vinyl label. This discolouration is not apparent in the vicinity of the anchor.

RESULTS

Laboratory Trial

Mortality within 1 week after tagging, and therefore possibly attributable to the tagging itself, was low (Table 1). Some other deaths were the result of mechanical problems, handling, and unspecified causes. Differences in mortality between the groups are not significant at the 5% level (contingency test).

Table 1. Results of laboratory tagging trials.

	Subcarapace tag	Abdomen tag	Carapace tag control
Die in week after tagging	0	1	1
Die before moult	16	10	13
Lose tag before moult	0	1	1
Moult, retain tag	15	13	0
Moult, lose tag	0	9	20
Unmoulted, tagged remainder	9	6	1
Total animals	40	40	36

Premoult tag loss was low. One tag was detached when a lobster crawled over a tank divider and, presumably, caused the tag to be pulled out.

There were no tag losses among the 15 moulted, subcarapace-tagged lobsters, whereas 9 of 22 abdomen-tagged animals lost the tag at moulting. These results are significantly different at the 5% level (χ^2 test).

Field Trial

Few lobsters below the legal size limit of $2\frac{1}{2}$ inches (63.5 mm) carapace length are normally landed at Miminegash. Tagged animals, however, have a reward value and are more likely to be landed regardless of size. In some instances, fishermen removed tags from short lobsters rather than land a sublegal animal. Because of this, groups above and below $2\frac{1}{2}$ inches carapace length when liberated are considered separately.

There were no returns from one group of 50 abdomen tags, which is significantly fewer ($Z = 3.26$, $P < .01$) than the next poorest group that had 9 returns from 48 lobsters liberated (comparison of two observed frequencies with the normal approximation (Brownlee, 1960, p. 161)). This implies a fault in the tags, their insertion, or in the condition of the lobsters. Because the fault cannot be ascribed unequivocally to the tag design, this group has been excluded from the analysis and calculations have been based on an abdomen tag release of 448 rather than 498.

Tag returns — In 1966 the subcarapace tags showed significantly higher returns of legal-when-tagged lobsters (162/338 = 48%) than did abdomen tags (86/289 = 30%) (Table 2) (comparison of two observed frequencies

with the normal approximation: $Z = 4.69$, $P < .001$). Returns of sublegal-when-tagged lobsters were not significantly different ($Z = 1.48$, $P = .14$). The returns of both tags were considerably lower than the 86% return of carapace-tagged lobsters released a few days before the opening of the 1966 season (D. G. Wilder, personal communication).

The 1966 40% return of subcarapace-tagged lobsters, legal when released, is significantly higher ($Z = 3.60$, $P < .001$) than returns of sublegal-when-released lobsters carrying the same tag (46/150 = 30%). Among abdomen-tagged lobsters the fractions returned are not significantly different ($Z = 0.52$).

There is a suggestion from the few tags returned in 1967 that the abdomen tag persists into the 2nd year better than the subcarapace tag. (The numbers returned were nine and eight respectively.) But only one lobster that was sublegal when released in 1966 was recaptured in 1967. There were no returns in 1968.

Movement — Points of recapture are known for 310 tagged lobsters returned in 1966. Most were taken within 2 miles of their release point (mean straight-line distance 1.8 miles). A few tags were returned by fishermen from nearby ports, including one from Cocagne, N.B., 30 miles southwest of Miminegash. The 1967 returns were all from the Miminegash area.

Incidence of moulting — In 1966, a significantly lower proportion of the returned abdomen-tagged lobsters had moulted compared with subcarapace-tagged lobsters (Table 3). The 1967 returns of legal-when-released animals are too small for statistical comparison; nevertheless, five of seven measured subcarapace-tagged lobsters had moulted compared with only one of the five abdomen-tagged lobsters for which both initial and final lengths were known. This corroborates the 1966 results, even though the numbers are small.

Growth at moult — With the exception of legal-when-released female abdomen-tagged lobsters that showed a significantly smaller mean carapace length increment than the corresponding subcarapace-tagged group (Table 4), the 1966 returns showed no significant difference between mean growth increments of any of the experimental groups. No particular reason can be suggested for the smaller growth increment of the abdomen-tagged females. The 1967 returns are too small to justify comparisons. There seems no reason to suggest that either tag contributed to a reduction in growth increment at moult. The increments are similar to those reported by Wilder (1953) for marked lobsters released in June at Tignish, P.E.I., and recovered a year later, but somewhat less than first moult increments reported for lobsters released in nearby Egmont Bay in September and October (Wilder, 1963).

Survival — It is possible to estimate a minimum survival figure for these sphyrion-tagged lobsters. A few days before the opening of each lobster season at Miminegash, 1000 lobsters are tagged with metal-elastic carapace tags (Wilder, 1954; Paloheimo, 1963). The proportion of these tags returned over the season is an estimate of the fishing mortality of the legal stock at the

TABLE 2. Returns of legal and sublegal sphyrion-tagged lobsters.

Size at release	Size at recapture	Year	Subcarapace tag			Abdomen tag		
			Released	Recaptured	%	Released	Recaptured	%
Sublegal	Known	1966	150	40	26.7	159	33	20.7
	Not known	1966		6	4.0		4	2.5
	Not known	1967		0	0		1	0.6
Legal	Known	1966	338	161	47.6	289[a]	75	26.0
	Not known	1966		1	0.3		11	3.8
	Known	1967		7	2.1		5	1.7
	Not known	1967		1	0.3		1	0.3
Not known[b]		1966		12	2.5		7	1.6
		1967					2	0.4
Total			488	228	46.7	448	139	31.0

[a]Excludes group of 50 tags for which there were no returns (see text).
[b]Returned lobsters not identifiable due to illegibility of tag.

TABLE 3. Numbers of known moulted and nonmoulted lobsters returned.

Location of tag:		Subcarapace			Abdomen			
Size	Year	Moult	Non-moult	% moult	Moult	Non-moult	% moult	χ^2
Sublegal	1966	33	7	82.5	21	12	63.6	2.44
	1967	0	0		0	0		
Legal	1966	72	89	44.7	22	53	29.3	4.43*[a]
	1967	5	2	71.4	1	4	20	1.37
Total		110	98	52.8	44	79	35.8	9.12**[a]

[a] $*P < .05$; $**P < .01$.

beginning of the season (i.e., excluding lobsters recruited during the season). The metal-elastic, carapace tag returns for the 1966 and 1967 seasons were 86 and 77% respectively (D. G. Wilder, personal communication).

The minimum survival value for legal subcarapace-tagged lobsters in the 1966 season (56%, Table 5) is significantly higher than for abdomen-tagged lobsters (35%, $Z = 5.4$, $P < .001$). The 1967 returns are too small to offer worthwhile comparison.

Survival of sublegal-when-tagged lobsters is impossible to calculate because some nonmoulted ones were probably returned directly to the sea. In addition, many of the smallest ones would escape from the traps before they were hauled. However, the returns in 1966 were quite low — between 23 and 30%, and only one was returned in 1967, which suggests strongly that there was mortality or tag loss.

TABLE 4. Initial carapace length in millimetres and increment at moult ($\bar{x}$) in mm and as a percentage of initial length of sphyrion-tagged lobsters released May/June 1966.

Size when marked	Year of return		Subcarapace		Abdomen	
			Male	Female	Male	Female
Sublegal	1966	n	17	16	11	10
		Initial length	59.6±2.3	58.9±2.8	59.2±3.0	59.8±2.5
		$\bar{x} \pm s$	8.3±2.0	7.9±1.7	8.4±1.7	7.7±1.5
		% growth	13.9	13.4	14.2	12.9
Legal	1966	n	21	51	11	11
		Initial length	69.5±3.7	71.1±5.6	70.6±5.9	71.7±5.3
		$\bar{x}$	9.5±2.0	10.2±1.8	8.7±2.2	8.4±1.9
		% growth	13.7	14.3	12.3	11.7
Legal	1967	n	2	3	1	–
		Initial length	83.7±0.4	77.8±9.6	85.0	
		$\bar{x}$	11.9±3.4	11.9±1.6	11.9	
		% growth	14.2	15.3	14.0	

TABLE 5. Survival of sphyrion-tagged, legal lobsters.

	Subcarapace tags	Abdomen tags
1966 returns		
Legal when released	338	289
Tag return	162	86
Metal elastic carapace tag return	86%	
Sphyrion tag survival	$\frac{162}{.86} = 188$	$\frac{86}{.86} = 100$
Survival to start of 1966 season	$\frac{188}{338} \times 100 = 55.7$	$\frac{100}{289} \times 100 = 34.6$

Injury — To give an indication of the possible incidence and extent of injury to internal organs among survivors, eight of the returned lobsters were killed and the tags dissected out (Table 6).

The subcarapace anchor is aimed at the relatively small anterior portions of the abdominal extensor of flexor muscles. The tissues in this region are soft and during tagging it is difficult to sense where the anchor is. Undoubtedly the chance of involving a vital organ is greater with this tag than with the abdomen tag and it is probable that the digestive gland is penetrated quite frequently. One lobster with digestive gland injury had moulted. The wounds of the five lobsters dissected were clean and not discoloured (Fig. 2).

The abdomen tag anchor is aimed at the large flexor muscles of the abdomen, but nevertheless, occasional injury to other organs, such as the gonad or posterior lobes of the digestive gland, is possible. One lobster with gonad injury survived. Without exception, the wounds and the musculature around the labels were darkened (Fig. 3), possibly a reaction with the vinyl label (Olsen and Fielder, 1968).

TABLE 6. Location of tag and condition of wound in eight dissected lobsters.

Tag		Relation to musculature	Other organs involved	Moult
Subcarapace	1	Tag lay lateral to muscles	Anchor through gill arch	No
	2	Anchor in right extensor	None	Yes
	3	Between extensor and flexor	Anchor lay against digestive gland	Yes
	4	Through extensor	Penetrated digestive gland	Yes
	5	Laterally and below extensor	Anchor against digestive gland (Fig. 2)	Not known
Abdomen	1	Through extensor into flexor	None (Fig. 3)	Not known
	2	Through side of extensor through flexor	None	Yes
	3	Through extensor into flexor	Gonad penetrated	Not known

DISCUSSION

The laboratory trial showed only that retention of the subcarapace tag at moulting was clearly superior. In the field trial, tag retention rates cannot be calculated but overall survival of subcarapace-tagged lobsters was significantly better than that of abdomen-tagged lobsters.

Factors that affect the estimated survival are natural mortality, mortality caused by tagging, movement of animals off fishing grounds, tag loss, and observation errors. Most lobsters were taken close to their release point and movement can probably be dismissed as a major source of error in estimating survival. Only one tag was returned by a buyer or processor suggesting that few escaped notice by fishermen; thus observation errors are probably low. Dr Richard Cooper (U.S. Bureau of Commercial Fisheries, Boothbay Harbor, Maine, personal communication) has used a similar sphyrion tag (back tag) on commercial-sized lobsters (81 mm carapace length and larger) off Monhegan Island, Maine, and by means of additional marks has established a tag loss rate for back (= subcarapace) tags of a little over 1% per month. Tag loss and mortality attributable to tagging of 450-g lobsters in the lab experiment were low, which suggests that mortality such as predation, if the lobster were disoriented or otherwise vulnerable following release, and natural mortality may have been the main causes of poor sphyrion tag returns off Miminegash. There is no a priori reason to suggest that the tags affect the catchability of the lobsters directly.

It follows that the overall differences between returns of the two experimental tags result most likely from a combination of lower survival and lower tag retention at moulting of abdomen-tagged lobsters.

It was expected that many of the tagged lobsters would have grown to legal size during their period at liberty and appeared in the catch. However, only one was returned in 1967 and none in 1968. In addition, the mean size at release of other lobsters that were recaptured in 1967 was considerably larger than that of the 1966 recaptures (Table 4), suggesting that mainly

larger lobsters survived a full year after release. This throws serious doubts on the effectiveness of either tag for long-term field studies with small lobsters. However, it should be pointed out that several of the early vinyl label sphyrion tags were successfully applied to lobsters as small as 30 mm carapace length. One of these retained the tag through four moults and measured 52 mm when it died, indicating that factors other than size may be critical.

Dr Cooper (personal communication) using a similar tag has had excellent results with lobsters 81 mm carapace length and larger released off Monhegan Island, Maine, in December, whereas similarly tagged lobsters released in July showed considerably poorer results. Wilder (1963), who released marked lobsters in September and October 1954 in Egmont Bay, P.E.I., calculated a survival rate of 76.4% to the start of the next season. This is higher than the minimum estimate of 56% for legal, subcarapace-tagged lobsters released off Miminegash in June. There is, therefore, a strong suggestion that lobsters released in summer will have a low rate of survival.

ACKNOWLEDGMENTS

I am indebted to Messrs G. E. Raine, U. J. Walsh, and A. J. Wilson for their assistance in the laboratory and the field. Dr D. G. Wilder kindly allowed access to information concerning the Miminegash fishery and, with Dr D. E. Aiken and Mr D. Iles, offered helpful criticism of the manuscript.

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Control of Infectious Hematopoietic Necrosis Virus Disease by Elevating the Water Temperature

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Studies were performed to determine if increasing water temperatures could control infectious hematopoietic necrosis virus (IHN) disease in sockeye salmon (*Oncorhynchus nerka*). Mortalities could be prevented if the water temperature was raised to at least 18 C within the first 24 hr after infection of the fish and if the fish were maintained at this temperature for 4–6 days. The disease did not recur after the elevated temperature treatment, but the fish would still contract the disease if they were reinfected. Reasons for the protecting action are discussed.

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INTRODUCTION

A VIRAL DISEASE, infectious hematopoietic necrosis (IHN), has impaired the successful rearing of sockeye salmon (*Oncorhynchus nerka*) at the International Pacific Salmon Fisheries Commission's (IPSFC) experimental hatchery at Cultus Lake, B.C. (Amend et al., 1969). Unless this disease can be controlled, it is unlikely that sockeye salmon can be reared successfully at this location.

Viral diseases cannot be controlled with standard chemotherapeutics. Consequently, the most successful methods of controlling viral diseases are immunization, serum therapy, and avoidance by regulatory methods. At present none of these methods can be used practically at this location.

Watson et al. (1954) showed that sockeye salmon infected with sockeye salmon virus experienced fewer mortalities when they were held at a water temperature of 68 F (20 C) than when held at 60 F (15.5 C) or lower. This paper describes experiments that were designed to test the effect of elevated water temperature on the control of IHN disease.

MATERIALS AND METHODS

The virus used in this study was isolated in 1968 from moribund sockeye salmon at the IPSFC hatchery on fathead minnow (FHM) monolayer tissue culture cells. Tissue homogenates (diluted 1:100) were filtered (0.45 μ) and 0.2 ml of filtrate were inoculated onto FHM cells grown in 2 ml of Eagle's minimum essential medium (MEM) containing 10% agamma calf serum and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). Fish examined for virus during experimentation were processed similarily. Clinical symptoms and the characteristic cytopathic effects (CPE) in FHM cells were considered presumptive evidence for presence of the virus (Amend et al., 1969).

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Experimental infections were induced in the following manner: (1) the IHN virus was first grown on FHM cells at 18 C and the infective medium removed from the cells after they showed extensive CPE; (2) the infective medium was diluted 1:100 with the hatchery water (approximate titer 1×10^4 $TCID_{50}$/ml, i.e., dilution causing CPE in 50% of inoculated tissue cultures); and (3) sockeye salmon were then infected with the virus at 12 C by placing the fish in the diluted medium for 30 min. This technique was used in all experiments except the first when the undiluted virus preparation was injected intraperitoneally into each fish.

EXPERIMENTAL PROCEDURES AND RESULTS

EXPERIMENT 1

To determine the minimum water temperature that would give protection to IHN-infected fish, undiluted infective tissue culture medium (0.05 ml/fish) was injected intraperitoneally into 160 twelve-gram sockeye salmon. The injected fish were immediately distributed into eight 5-liter cans with 20 fish per can. Two cans were placed at each of the following flowing (open system) water temperatures: 12, 16, 18, and 20 C (±0.5 C). In addition, duplicate control lots of fish at 12 and 20 C were injected with uninfected tissue culture medium. The various temperatures were obtained by heating dechlorinated Seattle municipal water of lower hardness to 20 C, and the intermediate temperatures were obtained by mixing with unheated water through a Powers mixing valve.

After 17 days at 12 C an average 68% of the fish were dead. About the same percent of fish died at 16 C, but the mortality was less at 18 and 20 C (Table 1). None of the control fish died. Although the number of deaths was

TABLE 1. Mortality of 12-g sockeye salmon 17 days after intraperitoneal injection (0.05 ml/fish) with IHN virus: effect of water temperature on mortality rate.

	Water temp (C)[a]							
	12		16		18		20	
Test group	Can 1	Can 2	Can 1	Can 2	Can 1	Can 2	Can 1	Can 2
Uninfected control	0/20	0/20	–	–	–	–	0/20	0/20
Infected	12/20	15/20	11/20	14/20	4/20	4/20	8/20	8/20
Avg % dead	68		63		20		40	

[a]Temperature range ±0.5 C.

less at 18 and 20 C, the disease was not completely controlled. These experimental procedures and results are similar to those reported by Watson et al. (1954).

EXPERIMENT 2

The method of infecting the fish in experiment 1 was unnatural and the results may not have any significance in a natural situation. Hereafter, all fish were infected with the virus simply by adding it to the water, and since

the elevated water temperature had a prophylactic effect, the increase in temperature will be referred to as treatment.

Fingerling sockeye salmon infected with IHN virus via water exposure show an increase in virus titer within 24–48 hr at 12 C and mortalities commence within 96–120 hr post-infection when the virus titer is at a maximum (about 1×10^6 $TCID_{50}$/ml of whole fish homogenate). As indicated in the previous experiment, mortalities could be reduced by increasing the water temperature, but it was not known if this would occur if the fish were not placed at the elevated temperature immediately after exposure. To determine the effect of a delayed temperature increase, 145 three-gram sockeye salmon were exposed to the virus at 12 C. At 0, 24, 48, and 96 hr after exposure, about 25 fish were placed and held continuously for 14 days at 20 C. One group remained at 12 C as an untreated control.

Mortality was completely prevented if the water temperature was increased from 12 to 20 C within the first 24 hr after infection (Table 2). However, if the treatment was delayed to 48 hr or more, it became progressively less

TABLE 2. Therapeutic use of continuous 20 C water temperature in controlling mortality due to IHN disease in 3-g sockeye salmon: effect of delayed treatment. Fish were infected via water exposure at 12 C and treated at 20 C. There were about 25 fish per group.

Group	Delay (*hr*) before temp raised to 20 C	14 day mortality (%)
1	0	0
2	24	0
3	48	30
4	72	61
5	96	72
Control	(Held at 12 C throughout)	72

effective. In fact, when treatment was delayed to 96 hr, the total mortality was the same as the untreated infected fish held at 12 C continuously. Apparently once the infection becomes established, a temperature increase has little effect on the course of the disease.

EXPERIMENT 3

Although IHN disease was prevented by placing infected fish at 20 C within 24 hr after being exposed to the virus, the prolonged rearing of salmonids at temperatures above 18 C is not advisable because of poor growth and the possibility of bacterial diseases. To determine the minimum time fish would have to be held at 20 C for the effect of increased temperature to control the disease, over 150 sockeye salmon (3 g) were exposed to the IHN virus at 12 C.

All fish, except for 25 maintained at 12 C as an infected control, were immediately placed at 20 C. Groups of 25 fish were then returned to 12 C 24, 48, 72, and 96 hr later. The experiment terminated 31 days after the fish were first exposed to the virus.

In the group of fish held continuously at 12 C, over 90% of the fish died within 14 days after being infected (Table 3). Increased water temperature had little effect on the total mortality if treatment at 20 C lasted only 24 hr. However, the effect was pronounced in the groups of fish held at 20 C for 48 hr or longer. The greatest effect was the delay of mortality. After 14 days the mortality in the 48- and 72-hr test groups was 17 and 0% respectively, but during the next 15 days the mortality rose to 61 and 38%. Deaths due to IHN

TABLE 3. Therapeutic use of 20 C water temperature in controlling mortality due to IHN disease in 3-g sockeye salmon: minimum treatment time. Fish were infected via water exposure at 12 C and treated for varying durations at 20 C, then returned to 12 C. There were about 25 fish per group.

Group	Treatment period (*hr*) at 20 C	14-day mortality (%)	31-day mortality (%)
Positive control	0	91	91[a]
1	24	71	71[a]
2	48	17	61[a]
3	72	0	38[a]
4	96	0	9[b]
Negative control	31 days	0	0[b]

[a]IHN virus isolated on FHM tissue culture.
[b]IHN virus not isolated.

virus was confirmed by reisolating the virus on FHM cells. The mortality in the 96-hr test group eventually accumulated to 9% after 31 days, but the virus could not be isolated from these fish and the reason for their death is unknown. Apparently a minimum of 96 hr at 20 C was necessary to prevent deaths due to IHN disease.

EXPERIMENT 4

The previous experiments showed that mortalities due to IHN disease could be prevented by increasing the water temperature and the disease apparently did not recur when the fish were returned to a lower temperature. However, it was not known how the fish were protected or the duration of the protecting action. To determine the length of time after treatment before the fish were susceptible to reinfection, sockeye salmon were exposed to the virus, treated, then reexposed at certain time intervals. In this instance fish (3.5 g) were infected at 12 C. About 30 fish were retained at 12 C as an untreated control, but all other fish were placed at 20 C for 6 days, then returned to 12 C. At this time 30 fish were placed in a separate can and not reinfected — a protected control. Another 30 fish group was immediately reexposed to the

virus after being returned to 12 C. Thereafter, an additional 30 fish were reexposed 6 and 24 days after the 6-day treatment. All fish were held at 12 C after reexposure and the mortality recorded for the next 21 days.

The duration of the effects of the heat treatment apparently is short because the fish immediately become reinfected after the 6-day treatment (Fig. 1). However, the fish became progressively resistant to reinfection the longer reexposure to the virus was delayed. For example, 77% died when the

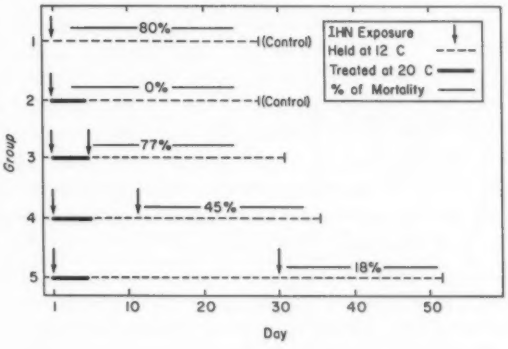

FIG. 1. Therapeutic use of 20 C water temperature in controlling mortality due to IHN disease in 3.5-g sockeye salmon: effect of 6-day treatment and susceptibility of fish to reinfection at various intervals after treatment. There were about 30 fish per group.

fish were reexposed immediately after the 6-day heat treatment, but only 18% of the fish died when reexposure was delayed 24 days. In comparison, 80% of the untreated controls died and none of the protected controls died. Possible reasons for the gradual increased resistance to reinfection after treatment may have been due to differences in challenge virus virulence, titer, or size difference in the fish, but other tests showed no such correlation. Furthermore, all environmental parameters remained constant. The fish, however, may have developed an immunity, but this was not confirmed.

DISCUSSION

Mortality due to IHN disease can be controlled experimentally but information was needed to determine if the disease could be controlled in a natural epizootic. The effect of elevated water temperature was tested by the IPSFC during an IHN epizootic in the summer of 1968 (J. Servizi, personal communication). In this instance mortality could be held to less than 10% if the water temperature was increased to 20 C immediately after diagnosis of the disease. However, if the temperature increase was delayed for about 1 week, the mortality was over 50% and if there was no temperature increase, all the fish died. In each instance the disease was confirmed by isolation of the virus on FHM cells, and the results compare favorably with those reported in this paper.

The reason for the effect of the increased temperature is unknown. The virus is unlikely destroyed at 20 C because it is routinely incubated in tissue culture at 18 C and can be stored up to 24 hr in distilled water at 28 C with

no decrease in titer (unpublished data). A more likely explanation is that the host is responding to control the virus. Perhaps increased hormone or enzyme activity or both are controlling the virus. It is not likely that an immune response is occurring because the fish are susceptible to reinfection immediately after treatment and the effect takes place too quickly to be an antibody response. A more probable explanation is that the fish are protected by interferon, a temperature-dependent, nonspecific anti-viral protein liberated by animal cells after some viral infections (Finter, 1966). Interferon prevents maturation of the virion, thus controlling the disease. Gravell and Malsberger (1965) presented evidence for an interferonlike substance in fish cells.

It is also unknown if the virus becomes latent after treatment only to recur at a later date, and if the protected fish become carriers or become immune. After these tests, I did not destroy any of the tested fish but held them continually near 12 C. I have not been able to reisolate the virus from these fish up to 16 months after the heat treatment. However, a few fish developed scoliosis, a common observation in populations of sockeye salmon surviving an IHN epizootic (Amend et al., 1969). At the IPSFC salmon hatchery, fish that received the heat treatment again contracted the disease 6 months later when they became ready to migrate to the sea (R. W. Mead, personal communication). Whether this represents reinfection from an outside source or evidence of a latent infection remains unknown because the carrier state of the virus has not been identified. This also indicates that either the fish were never immune or had lost their immunity. Therefore, more data should be collected before this technique is widely practiced, because it would be of no value if fish receiving the treatment became carriers to contaminate wild fish, or died later when they migrated to the oceans.

CONCLUSIONS AND RECOMMENDATIONS

Mortalities due to IHN disease can be controlled by raising the water temperature on infected fish to 18 C for 4–6 days, if the temperature is attained within the first 24 hr after infection. Care must be taken to prevent reinfection, because the fish are susceptible to the disease immediately after warmwater treatment. Anyone using this technique should be aware that the survivors may be carriers of the disease.

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Distribution of *Triaenophorus crassus* Parasites in Whitefish Flesh and its Significance to Automatic Detection of the Parasites with Ultrasound[1]

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FREESE, M. 1970. Distribution of *Triaenophorus crassus* parasites in whitefish flesh and its significance to automatic detection of the parasites with ultrasound. J. Fish. Res. Bd. Canada 27: 271–279.

The three-dimensional distribution of *Triaenophorus* infection in the whitefish (*Coregonus clupeaformis*) body and the ultrasonic echos received from the parasite cysts are studied in relation to the problem of detecting the parasites with ultrasound. It is shown that ultrasonic scanning of only 15% of the total surface area of a whitefish is required to illuminate (on the average) 70% of the total number of cysts in the body. As a result the search effort required for effective ultrasonic parasite detection can be greatly reduced. A considerably larger ratio of infection (3:2) for the right and left sides of the body is observed than has been previously reported. It is also shown that in the epaxial muscles a parasite is much more likely to be encysted near the surface than deep inside the flesh. Measurements of the ultrasonic cyst-echo durations yielded a distribution with two peaks of different amplitudes indicating a preponderance of spindle-shaped cysts as is actually observed. A negligible percentage of the echos in the distribution are less than 2 μsec in duration; the median width is 6.4 μsec. An outline is given of how these findings can be used to reduce the error rate in an automatic detection system. The paper concludes with a brief discussion of the effects of the transducer aperture on the latter distribution.

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THE DISTRIBUTION OF PARASITE INFECTION in fish populations must be known for the purpose of optimizing the performance of any given fish inspection procedure. The nature of these distributions in the case of *Triaenophorus crassus* infection of whitefish (*Coregonus clupeaformis*) is one of the principal factors affecting the efficiency of the ultrasonic parasite detection method described by Freese (1969). Equally important is the distribution of the parasites *within the host*. It is primarily this distribution that will be considered.

The morphology of the *Triaenophorus* parasite was given by Newton (1932) and Miller (1945, 1952) and need only be touched on here. The parasite is found encysted in the myomere of whitefish. The cyst volumes range typically between 0.03 and 0.12 cm^3 and come in a variety of shapes. Frequently the cysts are spindle-shaped, ranging from 6 to 25 mm in length and 3 to 4 mm in diameter. Normally only a single threadlike worm (up to 125 mm long)

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surrounded by a thick yellow fluid is found inside a cyst. In Fig. 1 are shown a cyst and a whitefish cross section containing a cyst.

The infection of whitefish populations as a function of age, weight, length, and locality has been thoroughly studied (Welch, MS, 1952; Miller, 1952; Lawler and Scott, 1954). It appears that for small fish (about 4–6 years in age) the infection distributions as a function of age are primarily of the exponential type. For older fish the distributions tend to the Poisson type; however, quite often they are widely divergent from a Poisson distribution with two or more peaks evident. Kennedy (1948) considered the statistical problem of sampling infected whitefish with such distributions. The relevance of these distributions to ultrasonic detector performance was emphasized by Freese (1969).

In contrast to this, data on the cyst distribution in the whitefish body was rather inadequate. For the purpose of analysing the cyst distribution, Newton (1932) had divided the body into just four parts. Welch (MS, 1952) had divided it into 14 parts — 7 on each side. Yet for our purposes this was still too coarse; moreover, the distribution of the parasites in a cross section of whitefish that is of importance not only to ultrasonic but also to candling methods was unknown. In obtaining this data it is possible to obtain another important parameter, namely, the median of the cyst-echo durations. The duration distribution is of course related to the dimensions, the shapes, and possibly preferred orientations of the cysts and the worms inside.

EXPERIMENTAL METHOD

The ultrasonic pulse-echo technique described by Freese (1969) was used to locate the parasite cysts inside a whole whitefish.

An ultrasonic transducer was used to project wide beams of ultrasound pulses at the whole fish. Both the transducer and the fish were immersed in water, which allowed the ultrasound to propagate without undue loss. The pulses were about 0.5 μsec long and had a carrier frequency of 5.4–6.9 MHz. In Fig. 2 the generation of echos by a cyst buried in the myomere is illustrated. Echos from inside the fish flesh were received with the same transducer and were electronically amplified. The echos were then examined for time-varying components caused by movements of the worm (Fig. 3). So that the fluctuations can be reliably detected (the fish itself must be rigidly positioned with respect to the transducer) the worm should move as much as possible. Experiments have shown that raising the cyst temperature "suddenly" from 34 F, which is normal for the iced fish, to about 55 F greatly stimulates the worm. Still higher temperatures increase the rapidity of the fluctuations only slightly and result in rapid fatigue of the worm. The fluctuations of the cyst echos have been studied in detail and are essentially random in character. A knowledge of the statistics that describe these echo fluctuations is of the greatest importance to the designer in selecting and predicting the performance of a given signal processing method; however, this subject will not be discussed here.

The depth of a cyst inside the flesh can be measured to within 1 mm — the theoretical resolution is approximately 0.4 mm at 6.5 MHz. The depth, s, in centimeters of tissue is obtained from the measured return transit time, t_r, by means of the formula $s = 1/2\ v_m\ (t_r - t_{sc})$, where v_m is the sound velocity in myomere. The return transit time through the scale-skin layer, t_{sc}, is roughly constant and equal to 1.0 μsec. The value of v_m (1.552×10^5 cm/sec at 50 F) has been obtained for whitefish by Freese and Makow (1968).

The determinations of the echo durations or widths were made by comparing the echo pattern for three successive photographs (taken at 20-sec intervals) and noting the extent of

VERTEBRAL COLUMN
PARASITE CYST

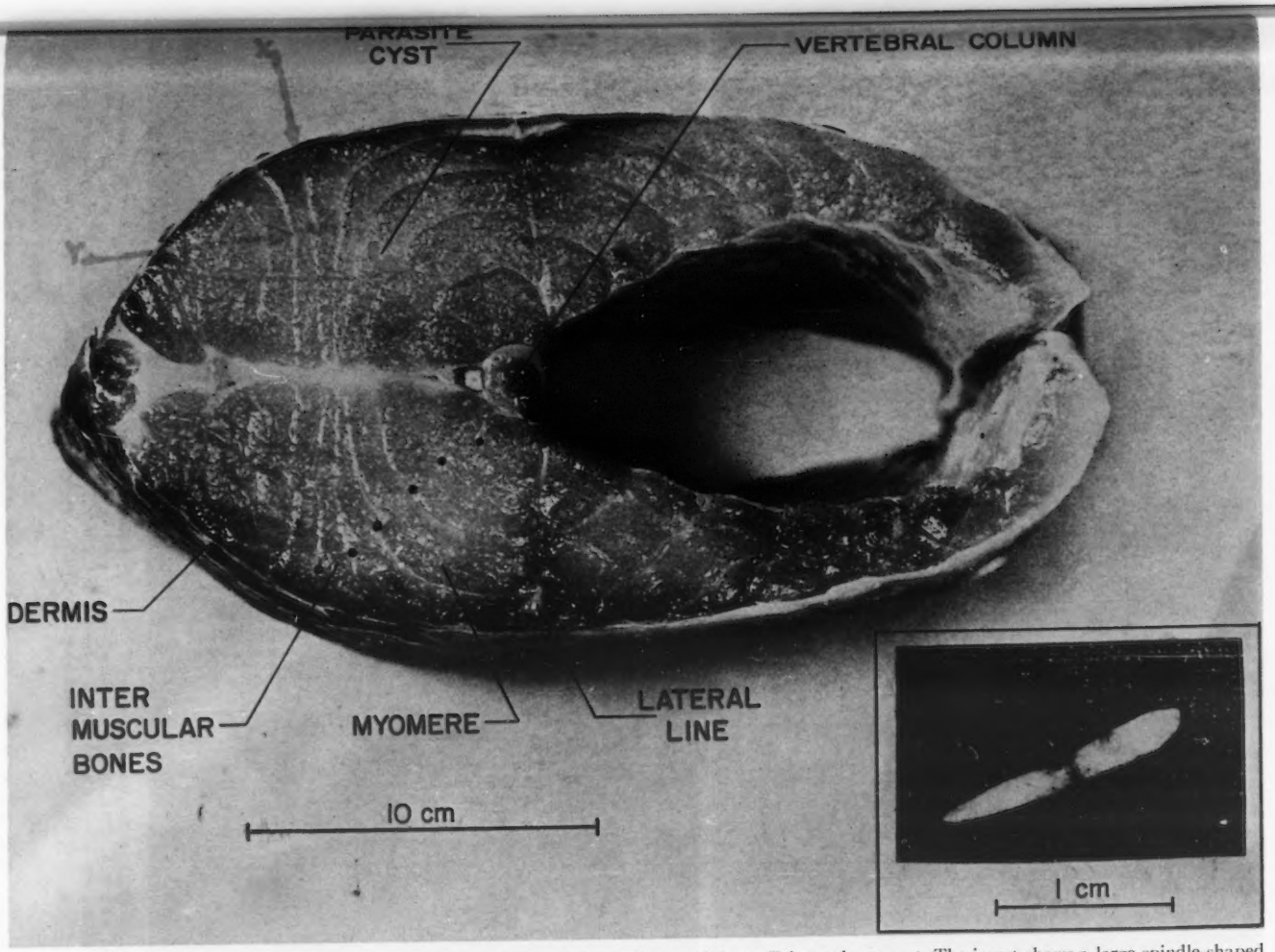

FIG. 1. Whitefish cross section in the region between the head and dorsal fin containing a *Triaenophorus* cyst. The insert shows a large spindle-shaped cyst.

Freese — J. Fish. Res. Bd. Canada

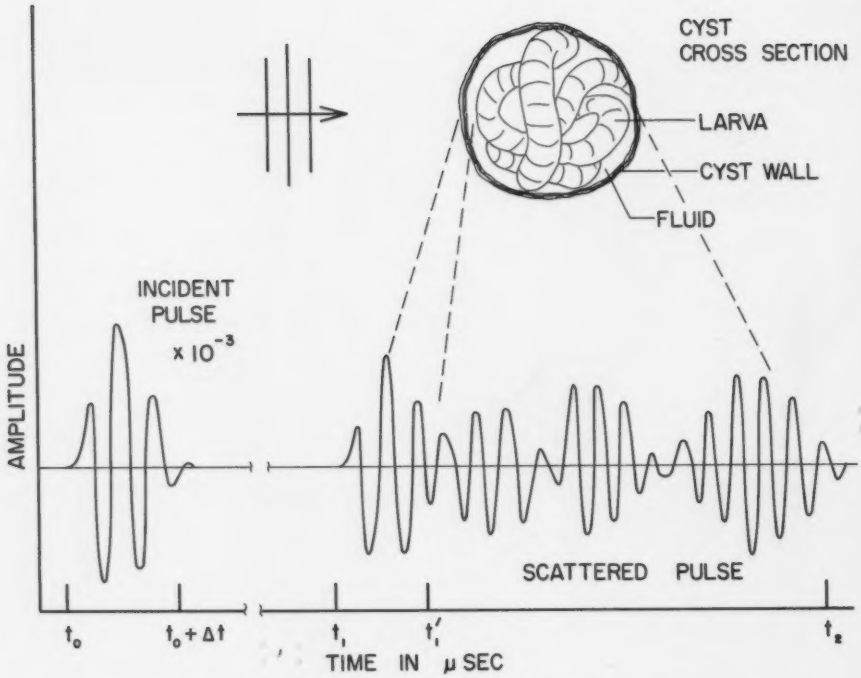

FIG. 2. Idealized cyst and cyst echo showing the origin of its various components.

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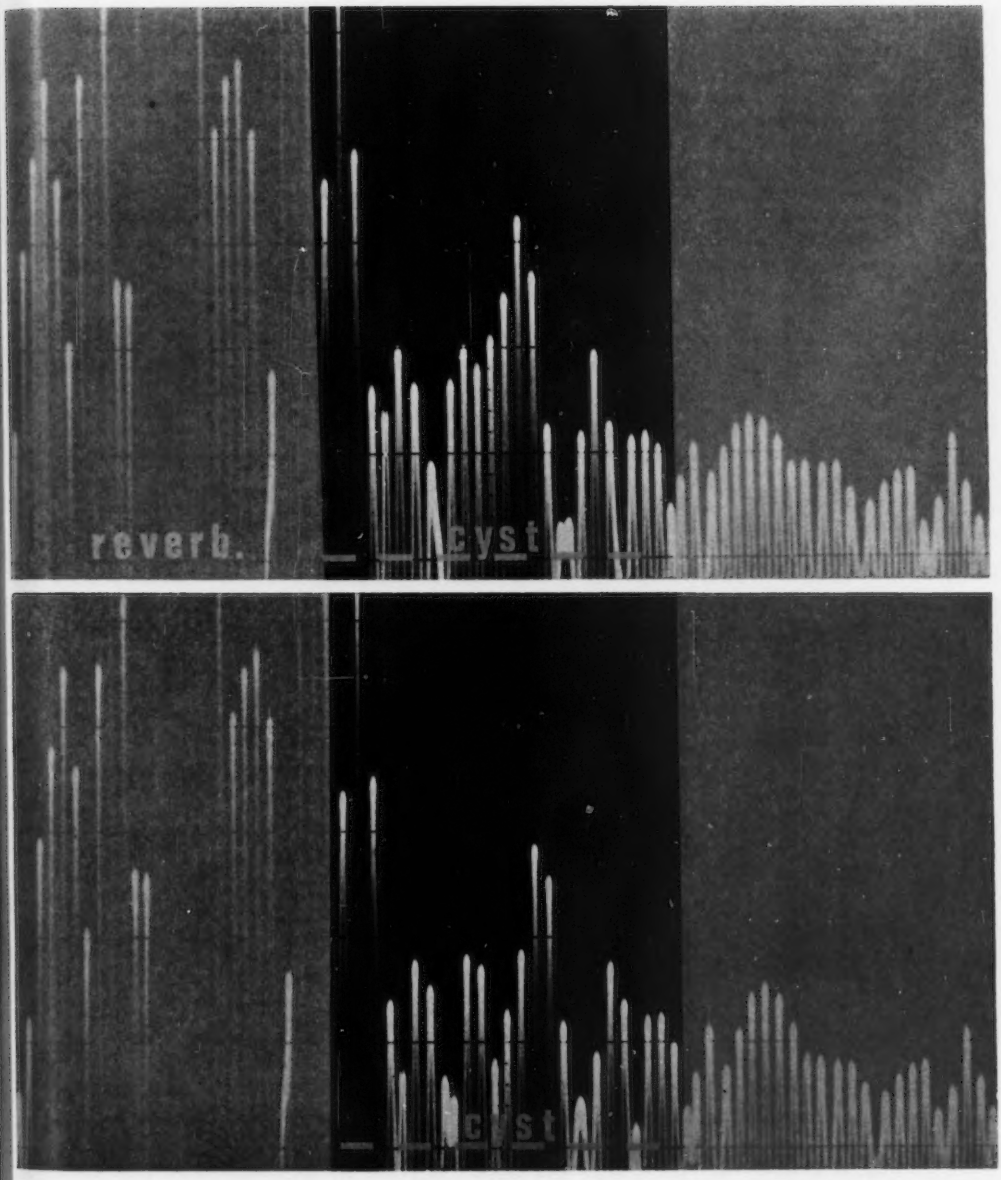

FIG. 3. A-scan pictures containing echos from a *Triaenophorus* cyst. When comparing the pictures, changes in echo structure (due to worm movements) are observed only in the specific region indicated. The time delay between these pictures was 5 sec. The region marked "reverb." is partially hidden by scale-skin reverberations. Sweep velocity = 1 μsec/cm; frequency = 6.9MHz; transducer diameter = 22 mm.

changes in the pattern along the A-scan axis caused by movements of the worm inside the cyst. For this reason, the echo width corresponds more to the extent of the coiled worm than to the exact cyst dimension normal to the ultrasonic beam. It is the echo width that is important in ultrasonic detection. The internal cyst echos result primarily from scattering by the worm to cyst–fluid interfaces and not from the cyst to host–tissue interfaces. Measurements of the densities of the various constituents (the densities and velocities appear in expressions for the reflection coefficients) and the frequency dependence of the scattering tend to confirm this. Density values are given in Table 1. The densities of the worm and the cyst fluid differ by a surprising 4.4%. The velocity in the cyst fluid appeared to be only slightly (1 or 2%) less than in flesh.

After ultrasonic scanning, the fish were dissected and the cyst locations were verified; where possible the cyst volumes and dimensions were recorded. All the ultrasonically scanned fish were part of the regular commercial run.

TABLE 1. Densities of *Triaenophorus crassus* cyst constituents and principal whitefish tissues.

Tissue constituent	Avg % composition by wt	Density at 20 C (g/cm^3)	Samples	SD (g/cm^3)	% accuracy
Larva	16	1.089	12	0.0041	0.5
Cyst membrane	10	1.067	3		0.25
Cyst fluid (for young cysts)	74	1.043	4		1.00
Cyst (whole)	100	1.053	7	0.0084	0.25
Whitefish myomere		1.055	21	0.0025	0.3

RESULTS

The infection distribution in the right side of a medium-large whitefish is shown in Fig. 4. This composite figure consists of the individual distributions from 12 fish taken from five Manitoba lakes and one Alberta lake. In a separate study of infection on the left side of the fish, the distributions of 11 fish from three lakes in Alberta, Manitoba, and Ontario were combined (see Fig. 5). Of the 15 fish actually considered in the first study, three contained cysts in the left side, but none in the right side. The regions of the epaxial muscle outlined by the grids in Fig. 4 and 5 were ultrasonically scanned and cover an estimated 13% of the total surface area on each side; each square measures approximately 3.6 cm^2. The cyst positions are indicated by dots in the figure. The distributions in Fig. 4 and 5 yielded the following infection percentages:

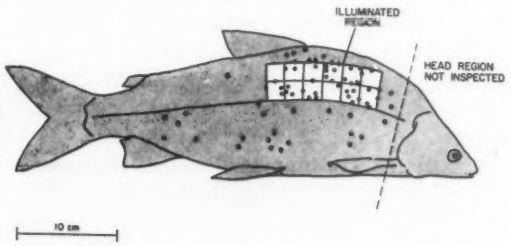

FIG. 4. Cumulative cyst distribution in the right side of whitefish. The grid marks the epaxial muscles that were ultrasonically scanned; the dots mark observed cyst locations.

Cyst location:	Epaxial region between the head and the trailing edge of the dorsal fin	Illuminated portion of the epaxial region (= 13% of total surface area)
Study #1 right side only	70.3%(52/74)	59.9%(44/74)
Study #2 left side only	83.8%(31/37)	67.6%(25/37)
Combined values for left and right sides	74.8%(83/111)	62.2%(69/111)

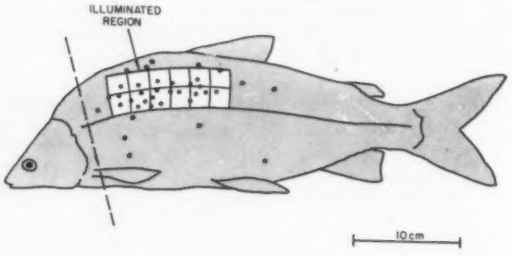

FIG. 5. Cumulative cyst distribution in the left side of whitefish. The distribution in this figure and those of Fig. 4 are the results of two separate studies.

These values would be decreased by 3–4 percentage points if it is assumed that 5% of the cysts occur in the head region as was indicated in Welch's data (MS, 1952) but this is of no great consequence. (The head region could not be examined because some of the fish were without heads.) The occurrence of five cysts above the anal fin in Fig. 4 is probably a coincidence because all five were found in a single fish. Otherwise the results seem to be in good order. Arbitrary partitioning of the right side sample into two groups, each group containing six fish, produced no great differences in the characteristics of the resultant distributions when compared with the original distributions. Also the distributions of cysts in the projected planes of the fish are in good agreement with the results published by Newton (1932) and Welch (MS, 1952). A similar procedure of subtracting the cyst contributions of two heavily parasitized fish, containing 13 and 24 cysts, respectively, indicated that the degree of infection (except perhaps very heavy infection of 30 cysts or more per fish) does not have much influence on the relative cyst distribution within the body of the fish as has been noted by other workers.

The above results show that the surface area of the fish that must be scanned by a parasite detector can be greatly reduced and still yield an acceptable detection efficiency; ultrasonic scanning of an entire fish would hardly be practical. These percentage distributions are, therefore, of great importance to ultrasonic parasite detection.

The ratio of infection in the left and right sides of the fish is of interest for the same reason. The values were:

	Right side	Left side
Study #1 (102 cysts)	67.6%	32.4%
Study #2 (90 cysts)	58.9%	41.1%
Combined result (192 cysts)	63.5%	36.5%

By itself this result would be of limited significance but a check of other cutting data (including heads) gave similar values. A further random sample from Goldsand Lake in Manitoba showed a ratio of exactly 60:40% for the right and left sides, respectively. This sample consisted of 200 highly infected fish containing 3300 cysts. Welch (1952) reported the percentage infections for two lakes. His combined data (355 fish and 1929 cysts) yielded a result of 54 and 46% for the right and left sides, respectively. However, it appears that on the average the right sides of infected whitefish contain about 50% more cysts than the left sides.

In Fig. 6 are shown the histograms of cumulative cyst counts on the right side as a function of myomere depth relative to the external scale surface. *The indicated depths of the echos in Fig. 6 correspond to their initial point of*

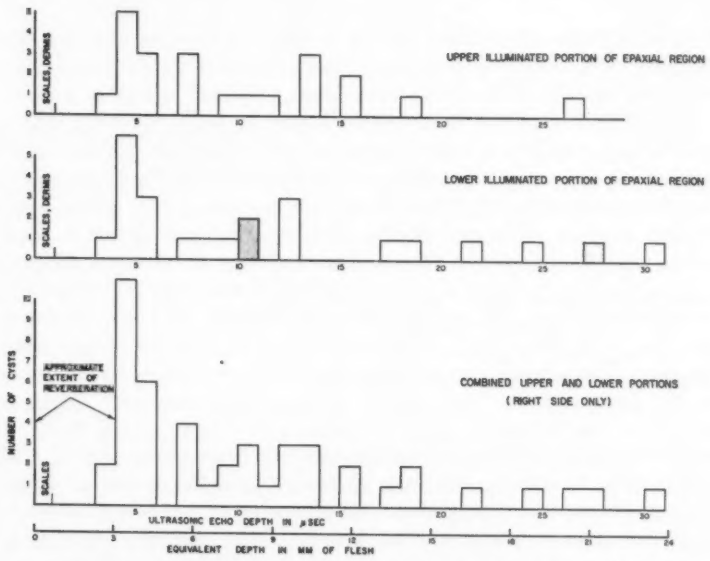
UPPER ILLUMINATED PORTION OF EPAXIAL REGION
LOWER ILLUMINATED PORTION OF EPAXIAL REGION
COMBINED UPPER AND LOWER PORTIONS
(RIGHT SIDE ONLY)
APPROXIMATE EXTENT OF REVERBERATION
SCALES, DERMIS
SCALES
NUMBER OF CYSTS
ULTRASONIC ECHO DEPTH IN μSEC
EQUIVALENT DEPTH IN MM OF FLESH

FIG. 6. Distribution of the number of cysts as a function of myomere depth in the portion of the epaxial region on the right side that was scanned. The echo depths correspond to the initial point of appearance of the cyst echos, not their centers.

appearance in the A-scan photographs. With the exception of two cysts, cyst counts in the first and second graphs of Fig. 6 correspond respectively to the upper and lower row of grid squares shown in Fig. 4. The beam-positioning grids do not extend to the dorsum because the depth of flesh becomes too shallow, and there are practical problems of poor beam-positioning geometry and beam overlap. The third graph of Fig. 6 shows the combined counts in the right side. A similar graph of combined counts in the left side (study #2) is shown in Fig. 7.

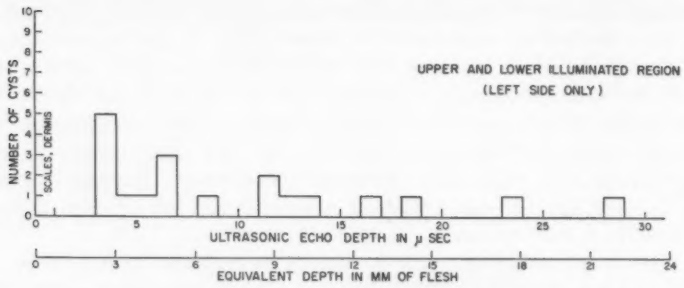
NUMBER OF CYSTS
SCALES, DERMIS
UPPER AND LOWER ILLUMINATED REGION
(LEFT SIDE ONLY)
ULTRASONIC ECHO DEPTH IN μ SEC
EQUIVALENT DEPTH IN MM OF FLESH

FIG. 7. Cyst distribution as a function of myomere depth in the epaxial region (illuminated portion) on the left side of whitefish.

Several features are evident in Fig. 6 and 7, the most obvious being the general trend of the cysts to be located close to the exterior surface of the fish. In the combined graphs, 50% of the cyst echos originate within 8 μsec (6 mm) of the surface and nearly 80% originate within 15 μsec (12 mm). This result, as graphs 1 and 2 in Fig. 6 clearly show, is not merely a reflection of the fact that the body decreases in thickness towards the dorsum. It must be emphasized that the ultrasonic echo depths refer to the beginning of a given cyst echo; the relation of these ultrasonic depths to the actual cyst depth is considered in another part of this paper. The sudden increase in the cyst frequency at 4 μsec is somewhat deceptive and is a result of the masking effect of scale-skin reverberation (see Fig. 3). Of the total number of cysts in the field of view on the right side, only three cysts were not detected ultrasonically; their positions, based on visual measurements, are indicated at 10 and 27 μsec in the second graph. The decline in cyst frequency with depth is thus not related to the decline in signal to noise ratio through the flesh. (In the first study the average depth of the myomere at the thickest part, measured from the midline to the exterior scale surface, was 2.50 cm (SD = ±0.18 cm). The estimated mean fork length of the fish was 41 cm. In the second study the fish were slightly smaller. The average depth to the midline at the thickest part was 2.28 cm (SD = ±0.020 cm) and the estimated mean fork length was 37 cm).

The tendency of the cysts to migrate to the surface will be of considerable benefit in the detection problem. Because of the poorer signal to noise ratio of the echos from deep inside the flesh, a higher detection threshold must be applied to these echos to decrease the probability of so-called type 2 errors.[2] However, as a result of the observed distribution, this will be possible without materially increasing the overall probability of type 1 error. In candling procedures generally, fillets from medium-large whitefish should be inspected more thoroughly from the surface side. In fact, for fillets approximately ¾

[2]Failure to detect the presence of a cyst is termed a type 1 error; type 2 error results when some echo is incorrectly identified as a cyst echo.

inches thick, about two to three times as much effort should be expended on the surface side.

In order that the ultrasonic depths, s, given in Fig. 6 and 7 may be correlated to the depths, d, based on visual determinations of the "geometrical center of gravity" of the cysts, the ultrasonically measured depths of the cysts in Fig. 7 have been plotted against corresponding visual observations in Fig. 8. The correlation coefficient of the values is 0.925. The depth, d, in millimeters may be determined to within about ± 2 mm using the resultant empirical formula $d = 0.63\ s + 4.06$ for $s > 4$ μsec.

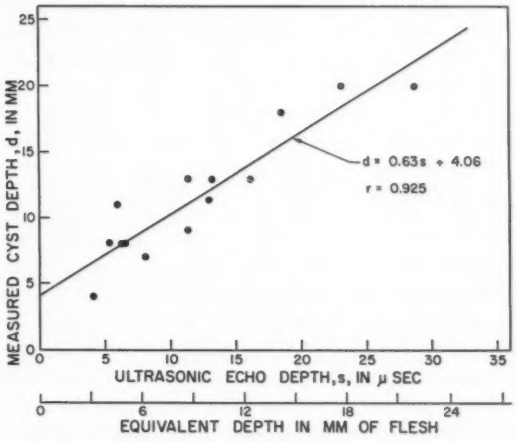

FIG. 8. Correlation of ultrasonic echo depths with visually determined depths of the "centers" of the corresponding cysts.

In addition to knowing the amplitude distribution of the cyst echos it is important to know the distribution of the echo durations. Two histograms of the number of cysts as a function of the echo width are shown in Fig. 9. The data were obtained with 22-mm diam circular transducers. Each cyst was counted only once although its echos might have appeared in more than one A-scan sequence, in which instance the average was taken. Plotting only those cysts completely illuminated (as far as could be ascertained) yielded the shaded histogram in Fig. 9. The histogram has two distinct peaks — at 6 μsec and at 10.5 μsec. The 6-μsec (4.5-mm) peak may be thought of as corresponding to roughly broadside incidence of the ultrasonic beam on an ideal spindle-shaped cyst and the 10.5-μsec (8.5-mm) peak as corresponding to nearly end-on incidence, the probability of which is much smaller. A somewhat larger proportion of longer echos ($\simeq$10–11 μsec) was observed in the left side than in the right side. This could indicate that a greater proportion of the cysts were oriented with their "longitudinal axis" normal to the surface. The average and median widths of the cyst echos are 7.1 and 6.4 μsec, respectively.

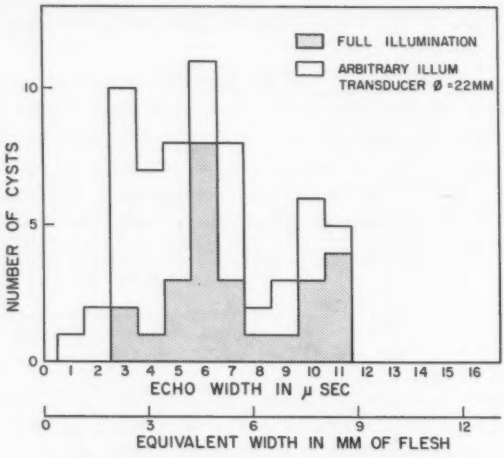

FIG. 9. Distributions of the cyst echo signal durations for total and "arbitrary" cyst illumination; transducer diameter = 22 mm; incident pulse length <1 μsec.

By also including those cysts that are only partially illuminated, a different picture is obtained. As might be expected, a substantial shift towards shorter echo widths is observed. The peaks are broader and not as clearly defined. The average echo width has decreased from 7.1 μsec for full illumination to 6.1 μsec and the median width from 6.4 to 5.8 μsec. Whereas in the former instance only 11.5% of the detected echos were less than 4.5 μsec long, for "arbitrary" illumination 31.8% of the detected echos are less than 4.5 μsec long. In practice, therefore, the observed widths of the cyst echos will depend on the transducer aperture. From the detection standpoint it is desirable to have as large and long an echo from the cysts as possible. However, no significant increase in average echo width will result once the aperture diameter exceeds the longest average cyst dimension (approximately 13 mm) by about a factor of three, only the reverberation noise will increase. The latter is generally undesirable and effectively limits the largest aperture that can be employed (Freese, 1969).

The negligible percentage of detected echos less than 2 μsec long provides a further means for reducing the type 2 error, which is perhaps the main source of difficulty in automatic parasite detection. Thus, an isolated detected signal occupying only a single 1 μsec range gate can be rejected regardless of its amplitude because of the very low probability that the signal is from an actual cyst. (This must be done before any significant threshold is applied to the detected cyst echos.) In this way type 2 errors can be significantly reduced without increasing the number of type 1 errors.

CONCLUSIONS

The distribution of *Triaenophorus* infection within the whitefish body has been more accurately determined. The distributions of cysts in the projected left and right planes of the fish are in good agreement with the

results published by Newton and Welch. However, the ratio of infection of the right side to the left side is considerably higher than the values Welch (1952) had obtained for fish from the Thunder Bay region. It is suggested that on the average the infection of the right side in whitefish is about 50% greater than the left side. The degree of infection (except perhaps very heavy infection) does not appear to have any significant influence on the relative cyst distribution within the body of the fish as has also been noted by other workers. These results confirm that the surface area of the fish that must be scanned by an ultrasonic parasite detector can be greatly reduced and still yield an acceptable detection efficiency.

It was generally found that *Triaenophorus* cysts tend to be located closer to the surface of the fish. Fully 50% of the cyst echos, in the portion of the epaxial muscles that was illuminated, originated within 8 μsec (6 mm) of the surface. The median duration of a cyst echo was found to be 6.4 μsec for full near-field illumination and 5.8 μsec for "arbitrary" illumination with a 22-mm diam transducer. The average cyst echo widths were 7.1 and 6.1 μsec, respectively. Only a negligible proportion of echos were of less than 2 μsec duration. These facts together with the previous finding may be used to reduce type 2 errors in an ultrasonic detection system.

ACKNOWLEDGMENTS

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Diel Vertical Movements and Feeding of Underyearling Sockeye Salmon and the Limnetic Zooplankton in Babine Lake, British Columbia

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NARVER, D. W. 1970. Diel vertical movements and feeding of underyearling sockeye salmon and the limnetic zooplankton in Babine Lake, British Columbia. J. Fish. Res. Bd. Canada 27: 281–316.

The use of a high frequency echo sounder and a midwater trawl in Babine Lake, British Columbia, in 1967 revealed a precise diel vertical movement of underyearling sockeye salmon. From early July to September, from about 1.5 hr after sunrise to 1.5 hr before sunset, the young sockeye were in two distinct layers at about 20 and 35 m, each layer about 6 m thick. About 1.5 hr before sunset the two layers began to ascend. About 0.5 hr after sunset all fish were within 3 m of the surface, and during darkness they were dispersed throughout the top 5–15 m with most fish being below the thermocline. Soon after the first light of dawn the fish usually tended to move toward the lake surface and then descended rapidly to the daytime depths.

By early October the pattern had changed markedly. During daylight the fish were still found in roughly two layers at about 24 and 40 m. However, the evening ascent did not commence until about 0.5 hr before sunset, the ascent was much slower, and the fish did not come to the surface but were dispersed between 9 and 27 m. This change in behaviour was temporally associated with a cooling of the epilimnion and a decrease in intensity of feeding.

Although the zooplankton of Babine Lake is composed of eight major species, only *Bosmina coregoni* and the calanoid copepod *Heterocope septentrionalis* displayed pronounced diel vertical movements. The former inhabited the surface waters during the day and were found in maximum abundance at 12–21 m at night. The latter was found at 21–30 m during the day and the surface waters at night. The major part of the zooplankton standing stock was found above 9 m.

Young sockeye fed most intensively in the evening as they approached the surface and again at dawn just as they commenced the descent. At those times the most common food item was *Daphnia longispina*, followed by *B. coregoni* and *H. septentrionalis*. These three species were strongly selected by young sockeye, since they were numerically much less abundant than other limnetic zooplankton species. In August, terrestrial insects were occasionally of major importance. At midday the upper layer of sockeye (about 20 m) was feeding on *H. septentrionalis* whereas the lower layer (about 35 m) was not feeding or was feeding at a low intensity.

The double layer of young sockeye during daylight occurred at least in the North Arm of Babine Lake and may be related to feeding. In July and August the fish in the upper layer had less food in the pyloric section of the stomach from 0900 to 1300 hr than did fish in the lower layer, whereas the volumes of food were similar in the two layers by late afternoon. Fish in the upper layer had much more fresh *H. septentrionalis* in the cardiac section from 1200 to 1800 hr than did fish in the lower layer. The ecological significance of the diel vertical movement is discussed briefly.

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INTRODUCTION

CERTAIN PHASES of the ecology of juvenile sockeye in the limnetic zone of nursery lakes have been reported in recent years, but the diel activity, including vertical movements and feeding habits, of young sockeye during the summer growing period has been generally ignored. Two-boat townetting techniques were developed in Alaska (Burgner, MS, 1958; Rogers, MS, 1967) and British Columbia (Johnson, 1956) for sampling young sockeye in the limnetic zones of large lakes; but this sampling was limited to a few hours of twilight or darkness when at least some sockeye were available to nets fished at the lake surface. In Lake Aleknagik, Alaska, a low frequency echo sounder revealed targets near the surface at night but not during the day, indicating a regular diel movement (Pella, 1968). In Babine Lake, Johnson (1956, 1958) was able to capture underyearling sockeye with a 1-m surface townet for only 0.5 hr (just after sunset) out of every 24 hr, although in a more recent paper (Johnson, 1961), using a 175 kHz echo sounder, he suggested that a few young sockeye may be also found at the surface at dawn. McDonald (1969) has shown that underyearling sockeye in Babine Lake are in the vicinity of the lake surface from evening dusk to dawn, when they can be captured by a large purse seine (18.3 m deep bunt), but they apparently are not near the surface during daylight hours as they cannot be captured at this time with this net. Furthermore, although it is well established that juvenile sockeye feed largely on planktonic Crustacea and may be quite selective (Ricker, 1937; Rogers, 1968; Hoag, MS, 1968), little is known about the time and depth of feeding in the lake. Thus the broad question motivating this research at Babine Lake in 1967 was simply: where are the fish during the diel period and what are they doing?

Preliminary work with an echo sounder in the south half of Babine Lake in 1966 suggested that underyearling sockeye perform rhythmic diel movements throughout the summer. In general, targets that I believed to be young sockeye were found at about 45 m during most of the day, rose to the surface at dusk (when young sockeye were captured by purse seine or townet), remained within about 15 m of the surface during hours of darkness, and returned to depth at dawn.

The objective of this study in the limnetic zone of Babine Lake in July to October 1967 was to describe the diel vertical movements of underyearling sockeye and to determine whether these movements were related to feeding by examining the vertical distributions of the zooplankton species and the feeding habits of underyearling sockeye.

STUDY AREA AND METHODS

Babine Lake (Fig. 1) is located in north-central British Columbia; it is about 160 km long and averages 3.2 km wide. A major feature of the lake is the physical, and to some extent the biological, distinctiveness between the two major sockeye nursery areas: the North Arm and the main lake regions. The North Arm has an area of 53 km^2, a maximum depth of 46 m, and an average depth of 20 m; in the main lake region these parameters are 437 km^2, 186 m, and 61 m

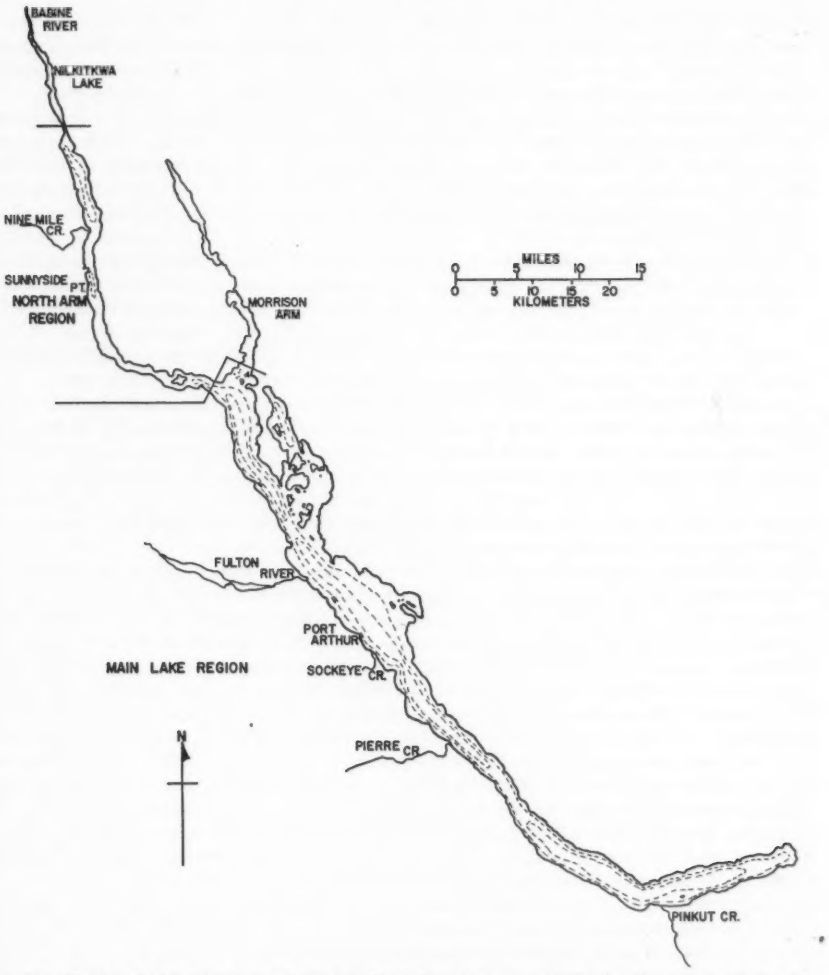


FIG. 1. Babine Lake, showing all geographical locations mentioned in the text and the 30- and 90-m lake contours.

respectively. The average density of underyearling sockeye in late summer is much higher in the North Arm than in the main lake, and the density of zooplankton is much lower (Johnson, 1961, 1964). The thermocline is generally sharper, shallower, and more persistent in the North Arm, which is rather protected from winds, than in the main lake, which is subjected to strong winds with a long reach (Johnson, MS, 1965a). The Secchi depth averaged about 0.5 m deeper in the main lake than in the North Arm in 1966. However, indices of primary productivity in the two areas were not markedly different (Narver, 1967).

Most of the observations in this study were made in the North Arm in the basin just north of Nine Mile Creek, although some observations early in the season were made near Sunnyside Point (Fig. 1). For comparative purposes some observations were also made in the main lake in the Port Arthur–Sockeye Creek and the Pierre Creek areas.

Field Methods

The field study was based on 24-hr series of samples and observations. The equipment used included an echo sounder, a midwater trawl, a purse seine, Miller zooplanton samplers, a thermistor thermometer, and a Secchi disc. Six series were conducted in midlake in the North Arm (one in the basin near Sunnyside, and five in the basin 3 km north of Nine Mile Creek), and three in the main lake (one 1 km offshore at Port Arthur and two 1 km offshore opposite Pierre Creek). The Nine Mile site was selected because it is the deepest basin in the North Arm; the main lake sites were selected because young sockeye could not be found in adequate numbers farther north. In general, the midwater trawling was conducted during the day and purse seining at night.

The echo sounder was a 175 kHz Sea Scanar (Honeywell) with a Model B1 dry paper recorder. Only vertical soundings were made in monitoring the vertical movements of fish or in confirming depth of sampling gear. The instrument was installed in a 6 m long outboard cruiser.

The midwater trawl was an Isaacs-Kidd model with a vertical opening of about 1 m, a codend 1 m long with 6 mm stretched mesh, and a body 2 m long with 12 mm mesh; it was operated from a 2.4 × 6.7-m barge powered by two 65-hp outboard engines. A hydraulic winch and davit arrangement (Andersen and Narver, MS, 1968) made handling of the trawl easy and rapid. Most trawling was done for about 10 min along a set course at about 9.7 km/hr (full power). It usually took 1–2 min to set and 2–3 min to haul the gear. The trawl was set approximately at the desired depth by a relationship between cable length, cable angle, and towing speed. The exact depth of the trawl was then determined by the echo sounder and any required adjustments in depth were made. Occasionally the depth was again checked at the end of a tow but little or no appreciable change in depth was noted.

Zooplankton sampling was conducted from the trawling barge using the same winch and davit arrangement. A 22-kg depressor was affixed to the end of the trawling cable and 3–5 Miller samplers (Miller, 1961) were attached at intervals (Andersen and Narver, MS, 1968). Each sampler had an 11.45 cm diam opening and nylon netting of 223 μ mesh with a screw-on plastic bucket. Tows were for 5 min at about 4.1 km/hr; depth of each sampler for a given amount of cable and cable angle at the standard speed was determined by the echo sounder. For work in the North Arm, samplers were placed at the following depths: 1, 3, 6, 9, 15, 21, 26, and 32 m; in the main lake they were placed at 1, 4, 7, 9, 17, 23, 32, and 45 m. In the North Arm 32 m was the maximum depth at which the sampler could be towed without danger of hitting bottom; in the main lake there was no such limitation. A current meter was located on the cable near the depressor providing a ready means of adjusting all tows to a common volume of water. Assuming that the Miller sampler passes water at 100% efficiency (Miller, 1961), the average volume filtered per sampler was 3.45 liters per 5 min (however, LeBrasseur et al. (MS, 1967) report the Miller sampler passes water at 140% efficiency).

The purse seine was operated as described by Durkin and Park (1967) from another 2.4 × 6.7-m barge powered by two 50-hp outboard engines. The net was 183 × 11 m with a 15-m long bunt; the lead had 1.9 cm stretched mesh and the bunt .6 cm.

The vertical temperature profile was taken once or twice during each 24 hr series with a thermistor thermometer that had a calibrated cable and a deck read-out. The relative transparency of the lake was measured at midday with a Secchi disc 30 cm in diameter.

Analysis of Samples

Zooplankton were identified from Ward and Whipple (1963) and from previous work done on Babine Lake zooplankton by Johnson (MS, 1965b). Zooplankton samples were made up to 100 ml and preserved in 3% formalin. Counting was done by splitting the sample to a $\frac{1}{8}$–$\frac{1}{64}$ subsample, depending on abundance of zooplankton. The subsample was distributed evenly in a gridded counting chamber of about 23 cm² area, and the organisms in each of five squares of 4 cm² were counted. These five squares were picked at random. The average count in the five squares of each species was then expanded to the 100-ml volume of the sample.

All fish (or subsamples from large catches) were measured after at least 24 hr preservation in 10% formalin. Up to 10 stomachs from fish in each of the middle three 5-mm length intervals

were taken for analysis. The cardiac (foregut) and pyloric (hindgut) sections were examined separately. All organisms were identified and counted, and a note made on relative degree of digestion.

Results for each sample were expressed by food item as percent occurrence and percent composition — a food item that ranked high in both was considered important. Percent occurrence of a particular food organism is the percentage of stomachs in which at least one individual of that organism occurs. Percent composition of a particular food organism is the average percentage of the estimated volume of a full stomach that is occupied by the organism (Thompson, 1959). In this scheme when the stomach section is opened the investigator makes an estimate of fullness using a six-stage scale of empty to full (0–25 food points). The food points (volume) of the stomach contents are then proportioned among the various food organisms in that stomach section. The points for each food organism in the sample are summed and expressed as a percent of the volume of a full stomach, i.e., percent composition.

Interpretation of Echo-Sounder Targets

A diel series of echo-sounder traces taken at Sunnyside Point, July 19–20, reveals some of the difficulties in interpreting targets (Fig. 2). During the day at 1200 and 0810 hr, targets occurred at 4–9 m and at depths over 15 m. In the evening (2005 to 2205 hr) the deeper targets appeared to move toward the surface whereas the shallower targets did not move appreciably. In the morning (0530 hr) some targets appeared to descend from the surface whereas others did not.

Presumably the targets that were at depth during the day and moved toward the surface in the evening were caused by rather different objects than were the targets that appeared at 4–9 m during the day. It will be suggested later that the daytime targets at 25 m and deeper are in fact from young sockeye whereas those at 4–9 m (a phenomenon that occurred only in early summer) are from zooplankton.

To test the reliability of the sounder in indicating the depth and perhaps density of young sockeye, 40 daytime tows with the midwater trawl were made at various depths and target densities in August in the Nine Mile Creek area. After the trawl was set and fishing, the echo sounder established the precise fishing depth. Then the echo sounding cruiser ran alongside and at the same speed as the trawl barge. The tows were made at depths ranging from 14 to 33 m and at target densities ranging from 3 per minute of running time to 78. Since the standard recorder paper was supplied with ruled horizontal lines every 3 m, it was convenient to count the upper end of each target that occurred in the 3-m stratum also containing the trawl. In addition, 18 simultaneous echo-sounder runs and midwater tows were made at depths ranging from 8 to 46 m at Pierre Creek on August 28–29.

The correlation coefficient of catch/minute against targets/minute was .81 for the 40 runs near Nine Mile Creek and .72 for the 18 runs at Pierre Creek (Fig. 3). These relationships suggest that this echo sounder is useful as an indicator of density and depth of young sockeye, at least during August. Thus an underlying assumption (although not vital to the argument) in examining echo-sounder traces in this study is that the targets are roughly proportional to sockeye density.

An area of possible interpretive difficulty with the Sea Scanar is that the transducer radiates a major lobe in a conical pattern having a beam width of 8°. This could cast doubt about the density of deeper targets relative to shallower targets since the horizontal area of the energy pulse would be about three times as large at a depth of 37 m (21.0 m^2) as at a depth of 21 m (6.8 m^2) (the approximate depths of the midday concentrations of young sockeye). The most daytime targets tend to be at the greatest depth. This concial pattern of the energy beam may not be a major problem, at least for descriptive work, as suggested by Fig. 3. It might even tend to be cancelled since the target strength decreases as a square of the depth. Of course, the high correlations might also be due to increased efficiency of the midwater trawl at increasing depths and decreasing light intensities.

I believe that daytime targets are mainly echos from schools or aggregations of young sockeye and not individual fish. In echo sounder traces from this study there tend to be more

targets at night than during the day (Fig. 2 and traces presented later). Presumably the light intensity after dark decreases below that required for schooling, the fish can no longer orient to each other, and they disperse.

The greater scatter of the points for late than for early August (Fig. 3) may be due to gear avoidance related to the increased size of fish. Average length of fish captured at Nine Mile Creek by purse seine on the night of August 5 was 52.7 ± .98 mm (2 SE; n = 570), and for the night of August 17 it was 56.9 ± .92 mm (2 SE; n = 607); fish at Pierre Creek on August 28 were at least as large as the latter but the purse seine was not used at night. As suggested later, the 1-m trawl may catch smaller fish than the presumably nonselective purse seine.

No fish other than underyearling sockeye were caught during the simultaneous echo sounder runs and midwater tows (Fig. 3). In fact, sockeye (including kokanee, the landlocked form of sockeye salmon) are by far the most abundant fish in the limnetic zone of Babine Lake. Rainbow trout, *Salmo gairdneri*, and lake whitefish, *Coregonus clupeaformis*, are the most common non-sockeye species, but in 30 purse-seine sets made at night from July 2 to October 2 only 10 and 11 individuals respectively were captured as compared with over 17,000 underyearling sockeye and 182 sockeye age I or greater. Most of the latter were presumably kokanee since over 99% of the smolts from the lake are yearlings that migrate in May and early June.

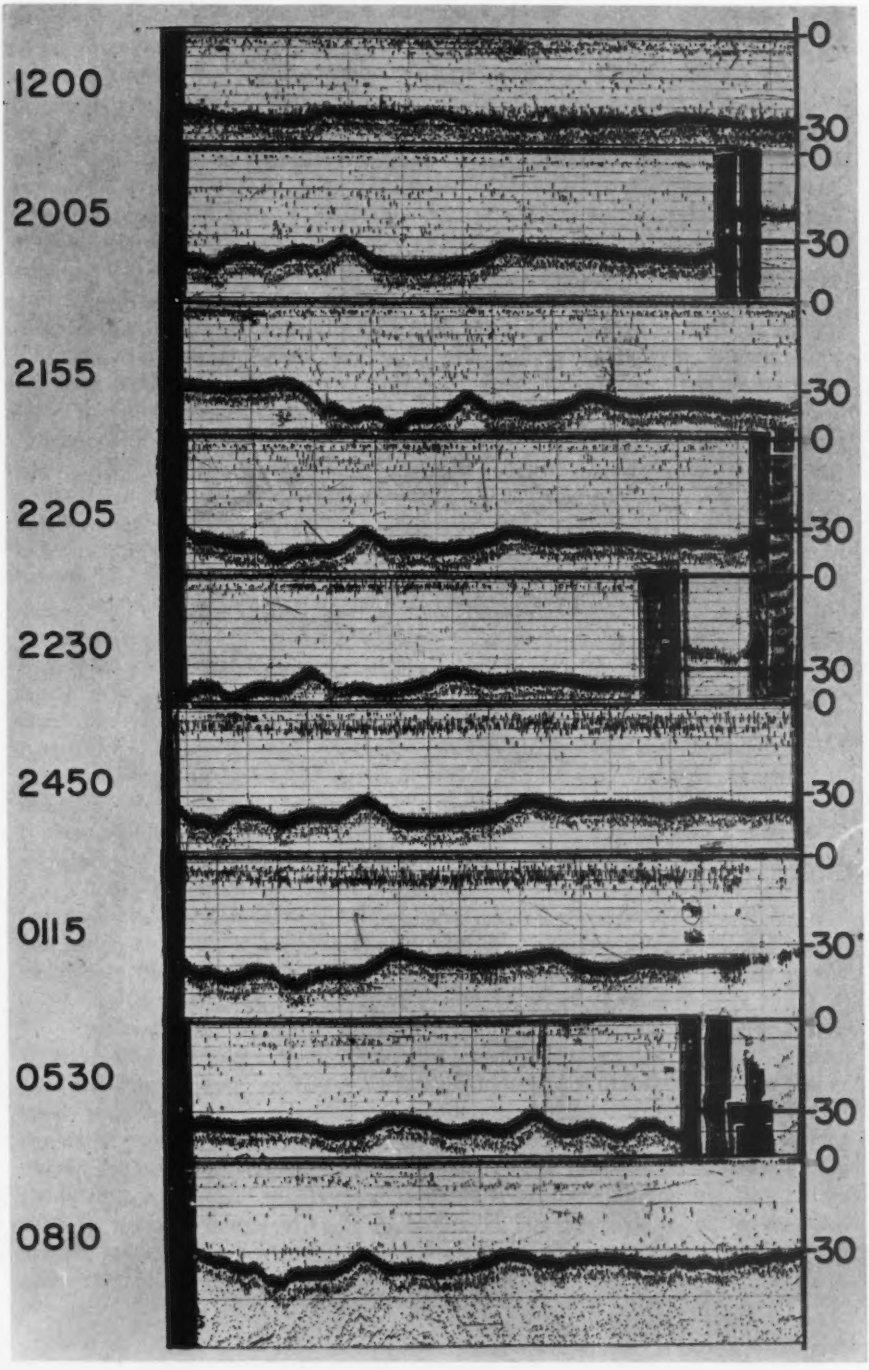
For the following reasons I attributed the band of dense targets in the 4–9 m stratum, that was fairly common during the day in early summer in both the main lake and the North Arm (e.g. Fig. 2), to a high density of zooplankton. The young sockeye are independent of the dense target band near the surface as they ascend in the evening and descend in the morning (Fig. 2). A major portion of the zooplankton standing stock as indicated by the Miller samples is found within 10 m of the surface and as will be shown later does not have a diel vertical movement. The near-surface band of targets was shown only by an echo sounder of relatively high frequency; the 175 kHz Sea Scanar detected daytime targets at this depth, whereas a 38.5 kHz Simrad did not. Both instruments recorded targets when young sockeye were as shallow as 9 m below the surface (Simrad information from J. G. McDonald), but a sounder with high frequency is presumably more likely to record echos from zooplankton (McNaught, 1968). Where this near-surface band of targets occurs, the zooplankton community is generally found by the Miller sampling to be much more dense near the surface than where these targets are not present. Daylight purse-seine sets made where these near-surface targets are dense catch virtually no underyearling sockeye and few other fish.

This band of near-surface targets may have been misinterpreted in earlier studies at the lake, and it may have led Johnson (1961), using the same echo sounder, to state that young sockeye in the lake during daylight hours are found almost exclusively at 0–5 m below the surface with a maximum at about 3 m. Such a behaviour of young sockeye was not observed in the lake during the summer and fall of 1967; during the day young sockeye were almost never found by the sounder or by nets above 15 m and they occurred as deep as 50 m. (In the later years of his study at Babine, Johnson (personal communication) recognized that young sockeye occurred at considerable depth during the day.) Another observation that may have led Johnson (1961) to his early conclusion about vertical distribution is that in some years extensive schools of young sockeye occur day and night in the littoral area of the North Arm until August.

DIEL VERTICAL MOVEMENTS OF YOUNG SOCKEYE

An overnight series of echo-sounder traces obtained on August 12–13 at the Nine Mile Creek Station in the North Arm (Fig. 4) shows the typical,

FIG. 2. A diel series of echo-sounder traces from a 2 km, north–south transect taken near Sunnyside on July 19–20. The lake surface is indicated at 0 m and the bottom by the wide dark band below 30 m. Black marks (targets) between the surface and the bottom are presumably from zooplankton, fish, or schools of fish. The true depth for a target is from the lake surface to the top of the target. The top 2½ m of the trace is blanked out because this is the approximate length of the firing pulse of the echo sounder. Sunset was at 2126 hr and sunrise at 0445 hr, and civil twilight (when the sun is about 6° below the horizon) was at 2217 hr and 0353 hr Pacific Daylight Time.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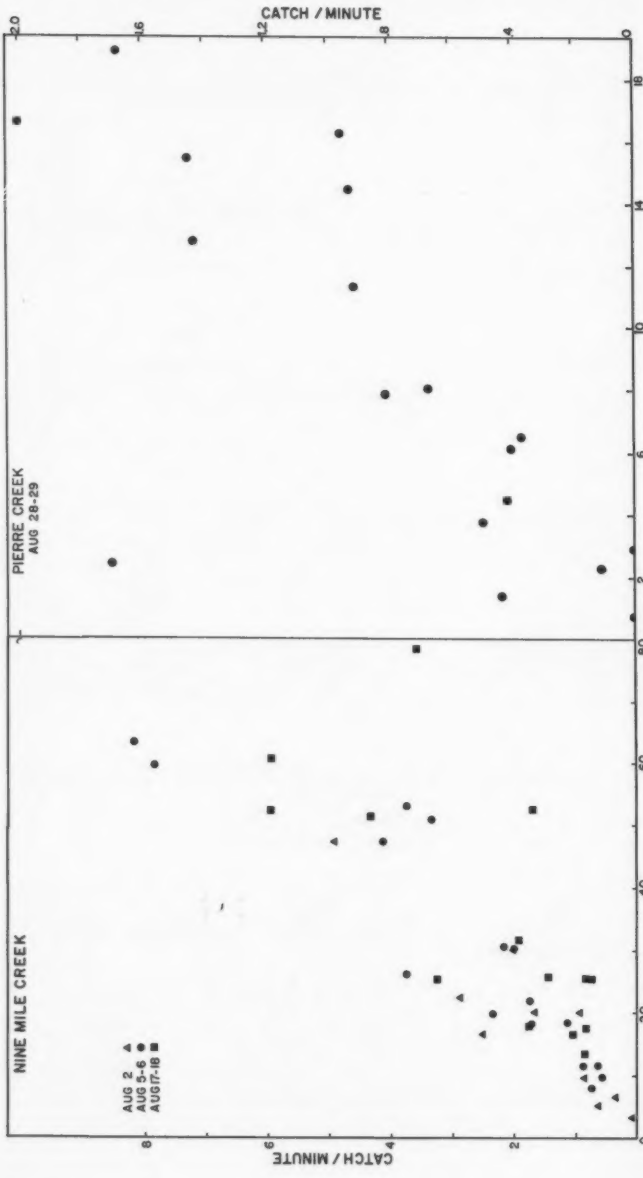


FIG. 3. Relations between numbers of underyearling sockeye salmon caught per minute of daytime midwater trawling and numbers of echo-sounder targets (in the 3-m strata that included the trawl) per minute of trawling near Nine Mile Creek (40 tows) and Pierre Creek (17 tows).

precise midsummer diel vertical movements of underyearling sockeye that were observed from mid-July into September. During this series the wind was 0–5 km/hr, the sky was clear, the half moon had set by 2230 hr, and the Secchi depth was 5.3 m. Through the hours of full daylight (1900 and 0800 hr in Fig. 4), sockeye were in two distinct layers, one at 17–22 m and the other at 27–36 m. In the early evening (about 2043 hr), the fish commenced to ascend while maintaining approximately the same distance between layers. The fish continued to rise until about 30 min after sunset (2200 hr), when they were almost all within at least 6 m of the surface. For about half an hour after civil twilight (2125 hr), fish were seen dimpling the lake surface (such dimpling was seen every calm evening through the summer; it varied in intensity, but generally not in time and duration relative to civil twilight). The total vertical ascent on August 12 occurred in about 2 hr. Within a few minutes after the completion of the ascent, the fish began to disperse away from the surface (2230 hr), but most remained within the top 15 m through the hours of darkness. At first light of dawn at least some of the fish again approached the lake surface (0430 hr; civil twilight 0445 hr), but the descent to daytime depths began soon afterward (0500–0530 hr). (Usually some fish can be seen dimpling the lake at dawn if the surface is calm.) During the descent the fish began to form the characteristic two layers (0530 hr), and by 0600 hr they were well formed. Within about 3 hr after commencing the descent (0800 hr), the fish reached their midday depths.

Diel series taken on a dark night (August 5–6, Fig. 5) and on a night with a full moon and a clear sky (August 17–18, Fig. 6) show the same general features as the series taken under a half moon that set before midnight and clear sky (Fig. 4). There is one difference in the vertical movements on August 5 and 17: the fish, just after midnight, seemed not to be dispersed as deep during the dark night as the one with a full moon. However, the vertical temperature profiles taken during the two series were also different; during the moonlight night of August 17 the surface temperature was 2 degrees C warmer, the thermocline at 10 C was about 4 m deeper, and the Secchi depth 1 m greater than during the dark night of August 5 (Fig. 5 and 6). The fish fed at night only under moonlight conditions (as suggested later). As any fish target that might occur in the top 2.5 m is not presented in Fig. 5 and 6 because of blanking by the pulse length, presumably fish were mainly in the 0–3-m stratum at 2142 and 0452 hr in Fig. 5 and at 2135 hr in Fig. 6.

Earlier in the summer the young sockeye during the day were somewhat shallower and the two layers were less distinct than in August. On July 19–20 at Sunnyside (Fig. 2) during full daylight (1200 and 0810 hr), the upper layer was 14–20 m deep and the lower layer 23–30 m. The fish ascended in the early evening (2005–2230 hr), remained mainly within 9 m of the surface during the hours of darkness (2450 and 0115 hr) and descended in the morning (0530 hr). Note that the bottom is shallower in the Sunnyside Point area than in the area north of Nine Mile Creek, but daytime sounding revealed similar vertical distribution of young sockeye on any date during the summer and early fall.

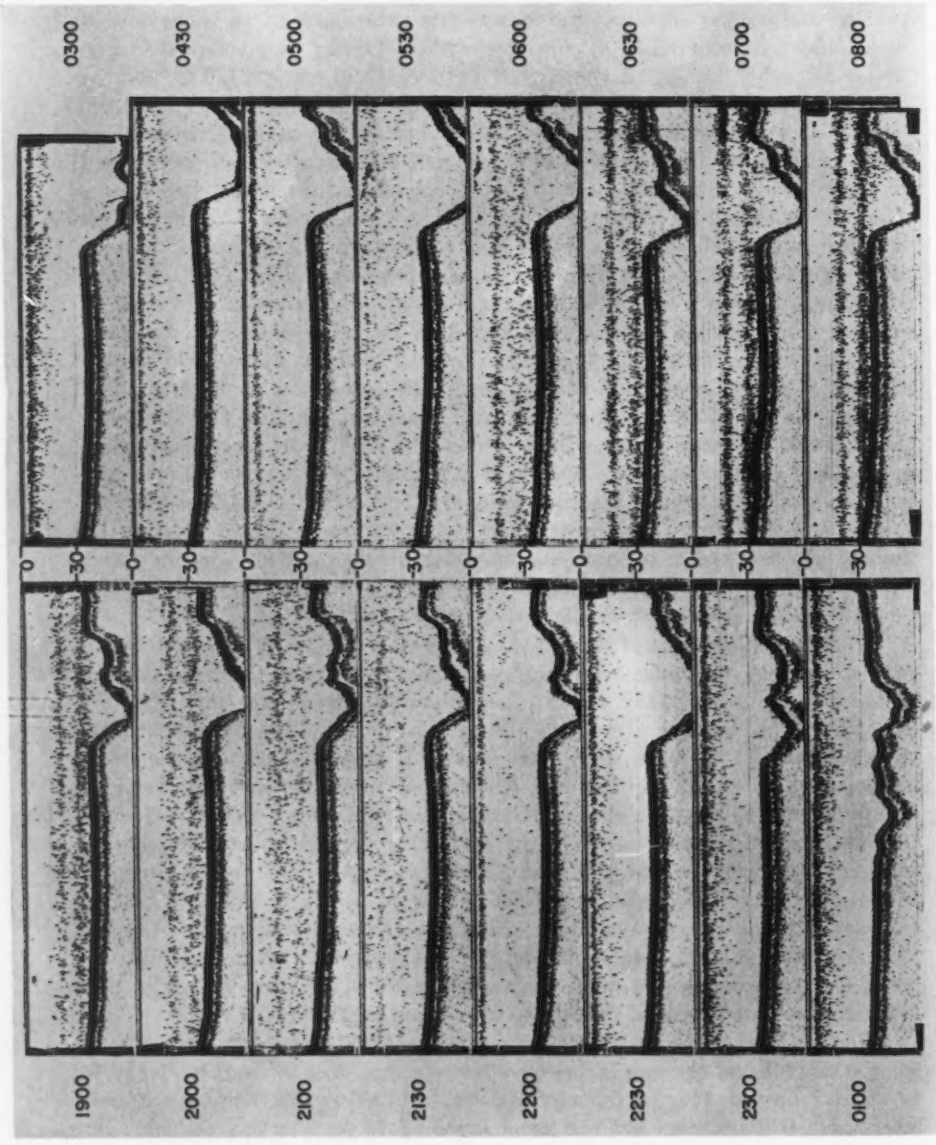
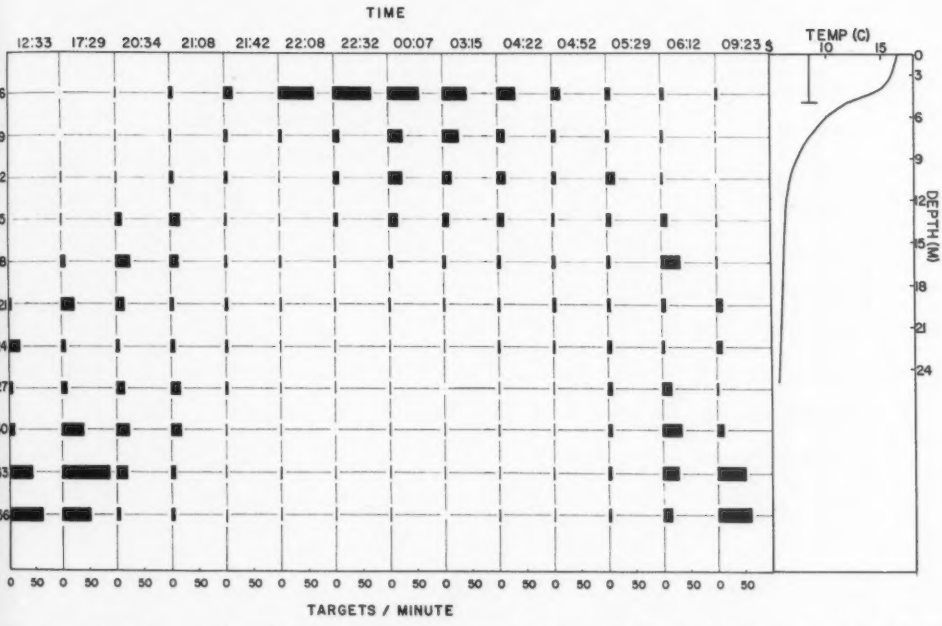


FIG. 4. An overnight series of echo-sounder traces from a 2.5 km, north–south transect taken north of Nine Mile Creek on August 12–13. Sunset was at 2043 hr, sunrise at 0527 hr, and civil twilight at 2125 and 0455 hr Pacific Daylight Time.

Major changes in the pattern of the diel vertical movement of young sockeye had occurred by early October. Unfortunately, the echo sounder was inoperative during the middle of September so the transition from summer to fall patterns of behaviour was not observed. On October 2–3 at Nine Mile in the



IG. 5. A graphical presentation of diel vertical movements of young sockeye and the thermal structure of the ke at Nine Mile Creek on August 5–6, a dark night. Bars are numbers of targets in each 3-m stratum per minute of nning (9.7 km/hr). Sunset was at 2055 hr, sunrise at 0515 hr, and civil twilight at 2140 and 0430 hr Pacific Daylight Time.

FIG. 4. An overnight series of echo-sounder traces from a 2.5 km, north–south transect taken north of Nine Mile Creek on August 12–13. Sunset was at 2043 hr, sunrise at 0527 hr, and civil twilight at 2125 and 0455 hr Pacific Daylight Time.

North Arm (Fig. 7 and 8), the young sockeye during the day still tended to be concentrated at two depths, but these layers were somewhat deeper and were less distinct than in August (Fig. 7 and in the morning in Fig. 8); at 0800–1315 hr the upper layer was widely dispersed from 18 to 27 m, whereas the lower layer was at a depth range of 32–40 m. It also appeared that fish in the upper layer (Fig. 7, 1200 and 1000 hr) may have been in larger schools than earlier since there are more large targets. The fish in the upper layer approached the surface at dusk and then descended, but fish in the lower layer ascended to only about 15 m (1900–2010 hr; civil twilight 1912 hr). Most of the fish spent the hours of darkness at 9–27 m, which is markedly deeper than during the summer (Fig. 4). The descent apparently started at least 30 min after civil twilight (0627 hr) since in Fig. 8 only a slight descent is revealed at 0702 hr. Both the ascent and descent were later in relation to civil twilight than observed in August.

Johnson (1958) reported that in October, when the surface temperature had decreased to 7 C, daytime surface catches of underyearling sockeye were greatly reduced. The change of behaviour in October that is documented here explains in part this marked decrease in catches. Surface temperature was about 12 C, the thermocline was at 10–15 m, and Secchi depth was 5 m.

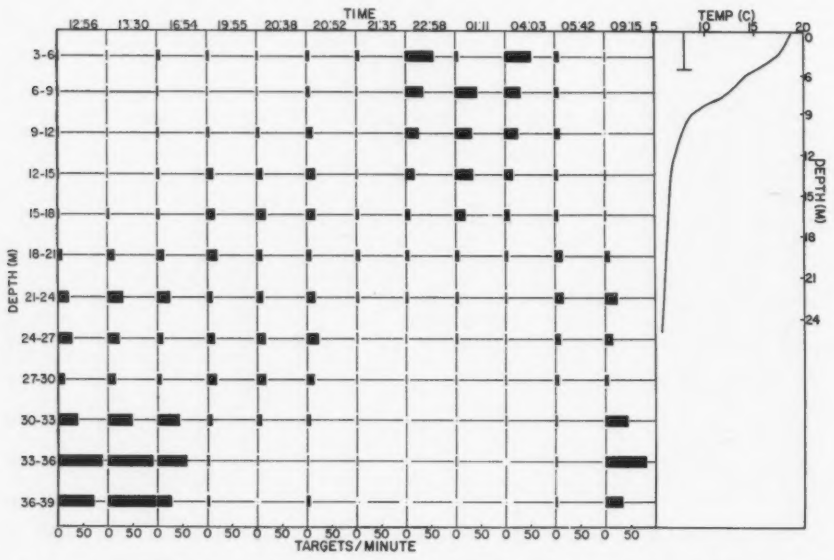


FIG. 6. A graphical presentation of diel vertical movements of young sockeye and the thermal structure of the lake at Nine Mile Creek on August 17–18, a night with full moon and clear sky. Bars are numbers of targets in each 3-m stratum per minute of running (9.7 km/hr). Sunset was at 2029 hr, sunrise at 0538 hr, and civil twilight at 2110 and 0455 hr Pacific Daylight Time.

Diel series conducted in the main lake on July 24, August 9, and August 28 revealed vertical movements similar to those described for the North Arm in midsummer. Fish were not as dense in the main lake as in the North Arm, so informative echo-sounder traces and adequate fish samples were more difficult to obtain. The August 28–29 series of echo-sounder traces taken off Pierre Creek (Fig. 9) can be compared with a series for the North Arm (Fig. 4). The wind was 0–6 km/hr, the sky was clear, a half moon was 20° above the horizon at 2400 hr, and the Secchi depth was 4.3 m. During the day the fish were found in two layers with the deeper layer much more dense than the shallower (1400 and 0900 hr). The two layers, at depths of 21–30 and 41–47 m, are about 6 and 12 m deeper respectively than in the North Arm in the latter half of August. This difference may be related to variables such as transparency of the lake and extent of cloud cover. In the late afternoon the two layers ascended at roughly the same rate (1710–2010 hr; civil twilight 2038 hr), until at dusk most fish had apparently reached the lake surface and were out of detection by the echo sounder (2040 hr). Soon afterwards the fish began to disperse throughout the top 15 m, where they remained during hours of darkness (2100–0110 hr). There is a suggestion in Fig. 9 that at least some fish moved towards the surface at first light of dawn (0440 hr; civil twilight 0529 hr) prior to descent. By 0900 hr fish were in two layers and were approaching depths they occupied at midday.

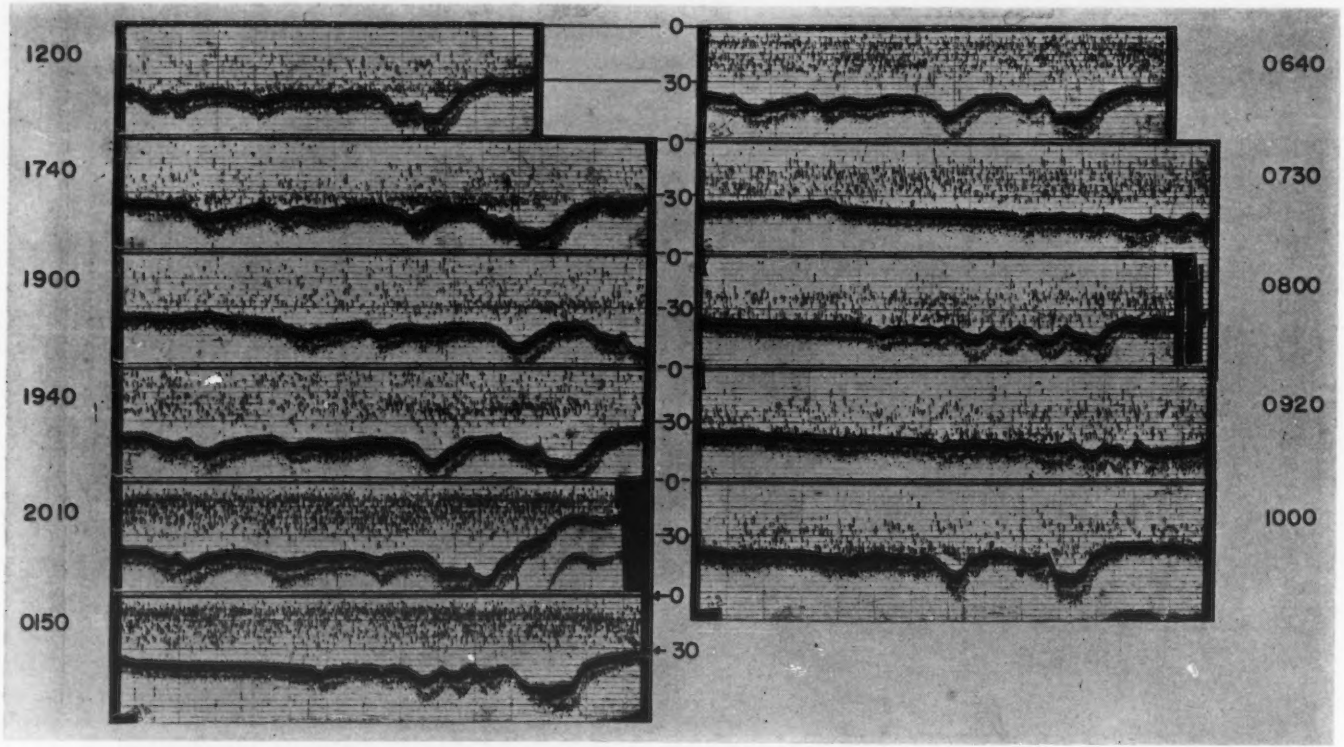


FIG. 7. A diel series of echo-sounder traces from a 2.5 km, north–south transect taken north of Nine Mile Creek on October 2–3. Sunset was at 1836 hr, sunrise at 0702 hr, civil twilight at 1912 and 0627 hr Pacific Daylight Time.

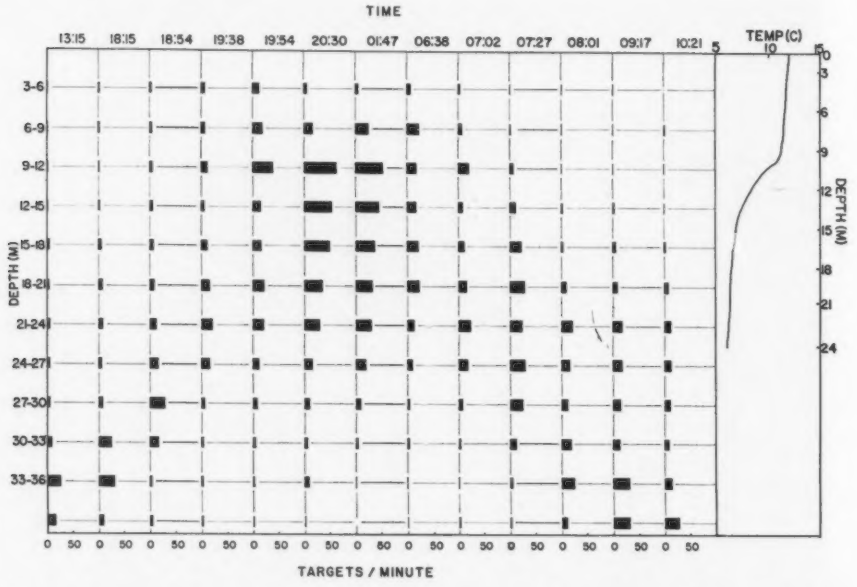


FIG. 8. A graphical presentation of diel vertical movements of young sockeye and the thermal structure of the lake at Nine Mile Creek on October 2–3. Bars are numbers of targets in each 3-m stratum per minute of running (9.7 km/hr). Sunset was at 1836 hr, sunrise at 0702 hr, and civil twilight at 1912 and 0627 hr Pacific Daylight Time.

On the basis of the echo-sounder traces the midday depth of fish increased somewhat from late July to early October in the North Arm (Table 1). This was temporally associated with increasing transparency since Secchi depth increased from 3.5 m on July 19 to 4.75 m on October 6 (Fig. 10). A similar increase in midday depth of young sockeye was noted in the main lake from late July to late August (Table 1). Although the midday depth of young sockeye in August was greater in the main lake than in the North Arm, the Secchi depth was slightly less in the main lake.

Using a Sea Scanar echo sounder and thermistor thermometer at Babine Lake, Johnson (1961) never observed young sockeye to descend through a well-defined thermocline. However, my observations suggest that the thermocline, within the temperature ranges that existed in July and August in the North Arm (Fig. 10) or in the main lake, presented no barrier to the diel vertical movement of young sockeye. Every 24 hr during these months most young sockeye ascended through the thermocline in the evening and after dark descended into or through it again. However, in October only fish in the upper layer approached the surface in the evening, whereas fish in the bottom layer ascended only to about 15 m and did not pass through the thermocline. This change in behaviour was temporally associated with a decrease in epilimnion temperature to 12 C and an increase in depth of the

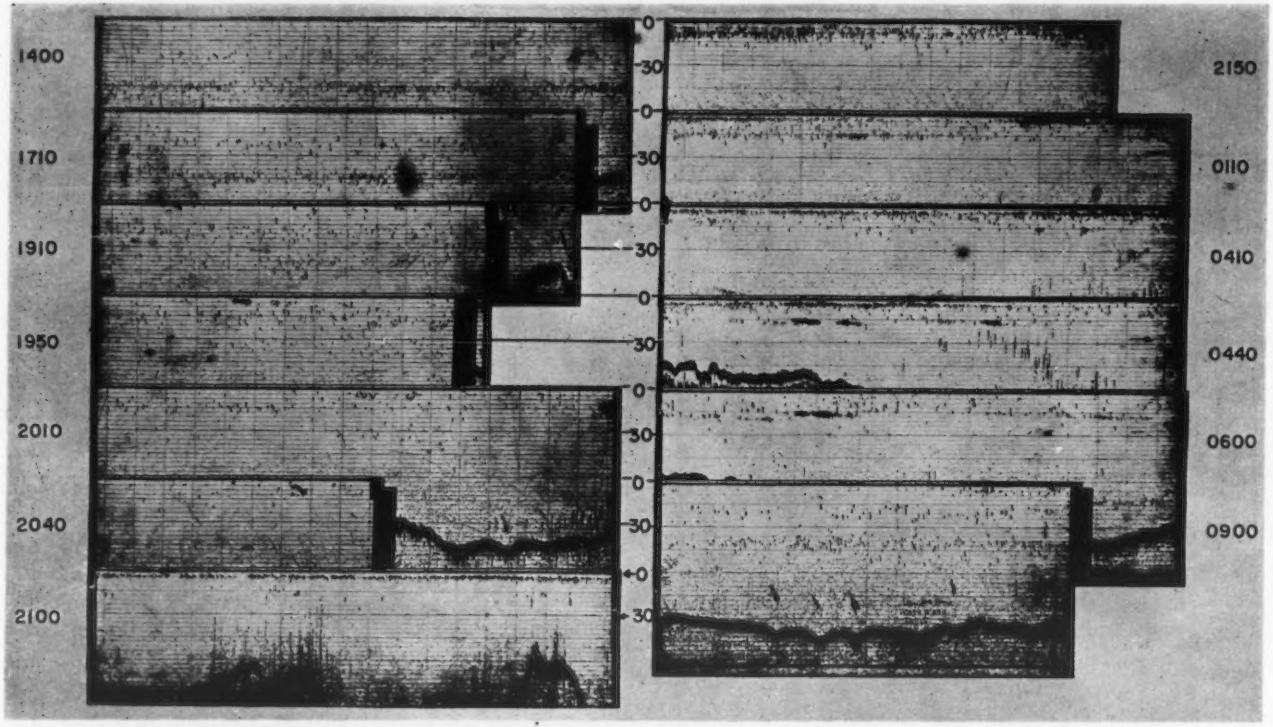


FIG. 9. A diel series of echo-sounder traces from a 2.5 km, northwest–southeast transect taken near Pierre Creek on August 28–29. Sunset was at 1958 hr, sunrise at 0603 hr, and civil twilight at 2038 and 0529 hr Pacific Daylight Time.

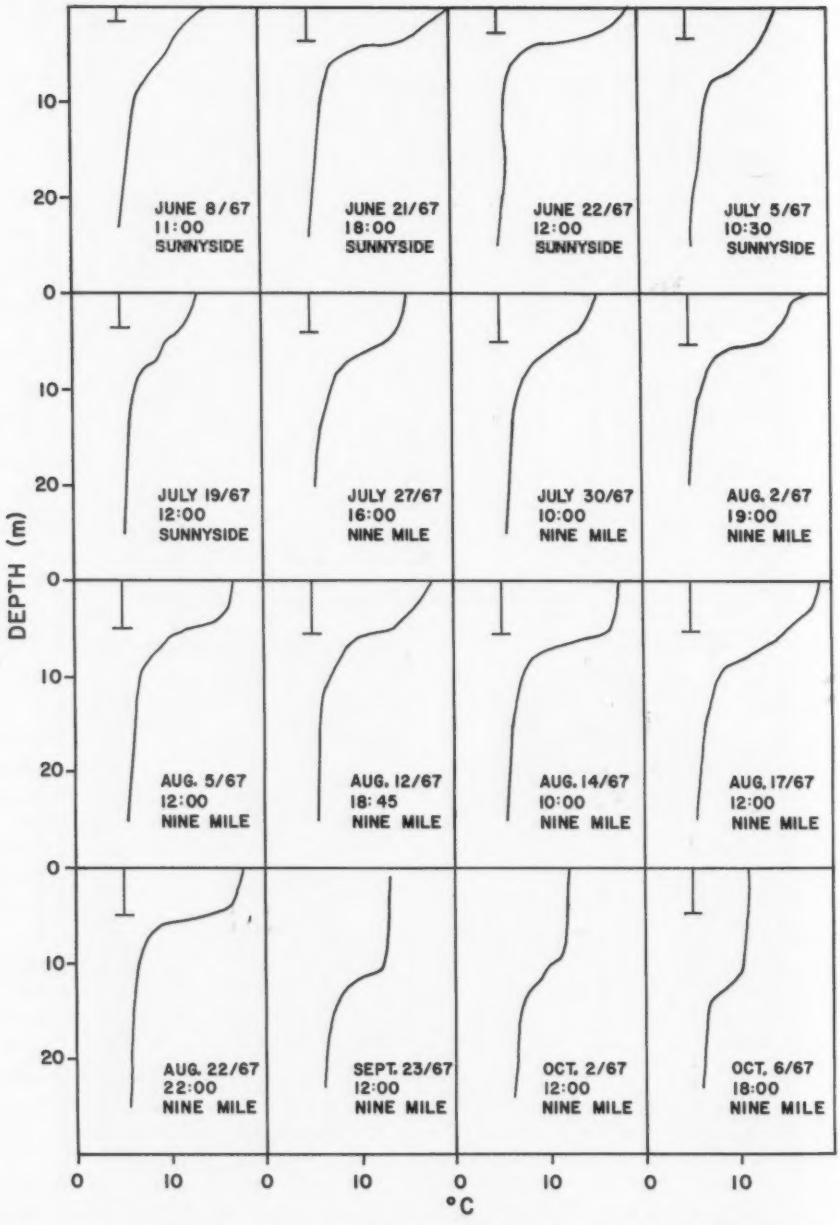


FIG. 10. Temperature profiles and Secchi depths in the North Arm for selected dates from June 8 to October 6.

TABLE 1. The midday vertical distributions of the two layers of age 0 sockeye in Babine Lake during the summer and fall as interpreted from echo-sounder traces, and the midday depth ranges of *Heterocope* as interpreted from Miller samples (Fig. 11 and 13).

Location	Date	Upper layer (*m*)	Lower layer (*m*)	Fig. no.	*Heterocope* (*m*)
North Arm	July 19–20	14–20	23–30	2	9
	27–28	15–24	27–37		6–18
	Aug. 5–6	18–24	30–38	5	21
	17–18	21–27	30–40	6	23–26
	Sept. 23–24	No sounder			26–32
	Oct. 2–3	18–27	32–40	7 & 8	23–32
Main lake	July 24–25	Not present	20–27		15
	Aug. 9–10	18–24	40–46		24
	28–29	21–30	41–47	9	29

thermocline (Fig. 8 and 10). There is also some evidence that the minimum depth to which the sockeye disperse after dark during the summer and early fall is influenced by the temperature and thickness of the epilimnion. During the middle of the night young sockeye, as indicated by Sea Scanar traces and temperature profiles, did not occur in large numbers in temperatures above about 12 C, although they moved into epilimnion temperatures higher than 12 C in the evening and the morning.

Considerable variability in mean length of young sockeye was revealed between gear, time of day, and depth (Table 2). Throughout July and August the mean length of young sockeye caught in any night purse-seine set was usually significantly larger ($\bar{x} \pm 2$ SE $\approx P = .05$) than the mean length of fish caught in any daytime trawl made in the same diel series (27 out of 31 daytime trawl catches; Table 2). In October none of the three purse-seine catches had a mean length that was significantly larger than any of the 10 daytime trawls. The mean length of fish caught by any night purse-seine set was, in two out of four comparisons, significantly greater than for fish taken by any night trawl made at a depth of 10 m at about the same time (Table 2). On July 19–20 and August 17–18 the mean length of fish taken by the single night trawl (10 m depth) was significantly greater than that of fish taken in most day trawls, but on July 27–28 the night catches tended to be about the same as day (Table 2).

A comparison was made of the mean lengths of 20 pairs of samples of underyearling sockeye taken during the day from the upper and lower layers by midwater trawl in July, August, and October in the North Arm (Table 2). In 17 of these pairs the mean length of fish in the upper layer was shorter (in 4 pairs the difference was significant at $P = .05$) and 3 were longer (none significant). A sign test (Natrella, 1963) for the paired mean lengths of upper and lower layer catches indicated that on the average the mean lengths of samples from the upper layer were smaller than those of the lower layer ($P = .05$).

TABLE 2. The mean lengths (mm) of underyearling sockeye captured by midwater trawl (IK) and purse seine (PS) in five 24-hr series made in the North Arm of Babine Lake in 1967. An X indicates that the mean length of fish in the upper layer (u) is less than that in the lower layer (l). A solid vertical line indicates statistical significance ($P = .05$).

Gear	Time	No. of fish	Mean	
July 19–20, Sunnyside Pt.				
IK-u	2045	11	38.09±2.10[a]	X
IK-l	2120	6	48.17±3.67	
PS	2150	22	45.91±1.73	
IK-10 m	2445	26	47.35±1.88	
PS	0150	114	47.97±1.10	
IK-u	0515	6	37.50±3.57	X
IK-l	0520	4	38.00±3.50	
IK-l	0930	6	41.00±2.06	
July 27–28, Nine Mile Cr.				
IK-u	1330	8	42.00±5.44	X
IK-l	1300	62	45.16±1.52	
IK-u	1715	7	37.71±5.51	X
IK-l	1650	56	42.71±1.80	
IK-u	2115	33	43.12±2.83	
IK-l	2100	22	42.68±2.73	
PS	2150	143	47.27±1.04	
IK-10 m	0120	9	42.56±5.16	
PS	0200	266	52.16±0.78	
IK-10 m	0500	18	39.28±2.55	
PS	0520	65	48.37±1.25	
IK-u	0910	2	44.00±8.00	X
IK-l	0845	23	46.65±1.82	
Aug. 5–6, Nine Mile Cr.				
IK-u	1200	26	45.65±2.13	X
IK-l	1245	118	47.30±1.36	
IK-u	1640	97	47.30±1.29	
IK-l	1730	44	44.84±1.31	
IK-u	2035	43	42.72±2.15	X
IK-l	2100	43	50.49±2.19	
PS	2200	169	51.53±0.92	
PS	2245	114	52.61±1.12	
PS	0130	117	52.36±1.17	
PS	0515	170	54.08±0.80	
Aug. 5–6, Nine Mile Cr. (*con't.*)				
IK-u	0640	28	48.93±2.33	
IK-l	0615	25	47.80±1.91	
IK-u	0920	17	47.00±2.75	X
IK-l	0845	137	48.47±1.13	
Aug. 17–18, Nine Mile Cr.				
IK-u	1330	29	51.14±2.37	X
IK-l	1300	68	51.22±1.86	
IK-u	1720	29	48.83±3.33	X
IK-l	1645	95	49.96±1.56	
IK-u	2100	11	45.82±5.47	X
IK-l	2040	14	56.14±2.52	
PS	2200	123	55.82±1.09	
IK-10 m	0110	124	56.37±2.83	
PS	0230	234	57.78±0.94	
PS	0500	250	56.53±0.83	
IK-u	0920	31	51.84±1.76	X
IK-l	0830	65	52.63±2.00	
Oct. 2–3, Nine Mile Cr.				
IK-u	1340	60	69.12±1.84	X
IK-l	1330	21	69.62±3.35	
IK-u	1820	26	68.19±3.92	X
IK-l	1845	3	72.00±2.00	
IK-u	2035	32	67.19±2.33	X
IK-l	2045	4	69.50±6.60	
PS	2200	236	67.47±0.95	
PS	0300	91	69.10±1.44	
PS	0600	156	69.50±1.04	
IK-u	0210	29	64.83±3.99	X
IK-l	0150	4	73.25±6.66	
IK-u	0930	6	68.67±6.10	X
IK-l	1000	15	71.73±3.19	

[a]Two standard errors of the mean.

That the mean length of fish from the upper layer was, on the average, less than that of the bottom layer might be due to a difference in gear avoidance at the two different depths. Successive purse-seine sets made on August 23–24 starting at 2030 hr (prior to the ascent of the first fish to within 10 m of the surface) and continuing until 0530 hr the next morning showed that the first catch at 2120 hr had a significantly smaller mean length than the subsequent sample taken at 2200 hr. The 2120 hr catch presumably consists mainly of fish from the upper layer whereas the 2200 hr fish are from both layers. This seems to suggest that in fact the fish in the upper layer are smaller than fish in the lower layer, but the mean lengths of subsequent catches through the night, when the fish from the two layers must be mixed, show considerable variability:

Time (*hr*)	2030	2120	2200	2315	0115	0310	0410	0530
No. of fish	5	178	214	224	171	221	240	196
Mean length (*mm*)	59.6	57.6	60.6	58.2	58.7	56.0	59.0	58.2
Two standard errors of mean	6.08	1.00	0.90	0.82	0.92	0.88	0.86	0.86

(civil twilight was at 2102 and 0510 hr)

COMPOSITION AND DIEL VERTICAL DISTRIBUTION OF ZOOPLANKTON

The species of zooplankton that occurred commonly in the Miller samples were: the calanoid copepods *Diaptomus ashlandi*, *D. pribilofensis*, *Epischura nevadensis*, and *Heterocope septentrionalis*; the cyclopoid copepod *Cyclops scutifer*; and the cladocerans *Daphnia longispina*, *Bosmina coregoni*, and *Holopedium gibberum*. The zooplankters from lowest to highest in relative abundance in early August in the North Arm were *H. gibberum*, *D. longispina*, *E. nevadensis*, *H. septentrionalis*, *B. coregoni*, *D. pribilofensis*, *D. ashlandi*, and *C. scutifer*. Chironomid larvae occurred infrequently in the zooplankton samples.

The abundance of most species of zooplankton in the North Arm changed during the period of June 8 to October 2 (Table 3). Only *C. scutifer* showed no obvious fluctuation in abundance. *Diaptomus pribilofensis*, *E. nevadensis*, and *H. septentrionalis* were apparently not present in the North Arm as adults or late copepodids on June 8 but appeared later in June. Both *D. ashlandi* and *D. pribilofensis* decreased after June 21–22, the former by a factor of about 2 and the latter by about 12. *Daphnia longispina* increased in June and early July but by October had decreased to a low level. *Bosmina coregoni* showed a similar early summer increase followed by a low density in mid-summer and another increase in September. The abundance of *H. septentrionalis* increased slowly to mid-August and then decreased in the fall. (Identification errors may have occurred in the June 21–22 samples between the early copepodid stages of the related calanoids *E. nevadensis* and *H. septentrionalis*, and may have resulted in too high an abundance of the former.)

TABLE 3. Indices of abundance of zooplankters (mean total catches of 7 Miller samplers towed horizontally for 5 min at standard depths to 26 m) for all diel series in the North Arm of Babine lake in 1967. Only day samples are used for *Bosmina coregoni* and only night samples for *Heterocope septentrionalis*. Both day and night samples are used for the other species.

Date	Location	*Diaptomus ashlandi*	*Diaptomus pribilofensis*	*Epischura nevadensis*	*Heterocope septentrionalis*	*Cyclops scutifer*	*Daphnia longispina*	*Bosmina coregoni*	*Holopedium gibberum*
June 8	Sunnyside	118,600	0	0	0	56,600	1,700	4,700	0
21–22	"	111,500	28,500	5,100	700	50,200	4,300	10,200	300
July 19–20	"	58,200	15,300	20	2,700	72,600	9,500	44,700	40
27–28	Nine Mile	28,900	12,300	90	2,900	42,300	2,700	1,700	80
Aug. 5–6	" "	38,600	8,900	3,000	4,400	61,500	1,600	5,600	50
17–18	" "	27,500	9,300	1,200	4,900	57,700	1,400	8,500	150
Sept. 23–24	" "	62,800	4,500	420	1,300	59,000	2,700	20,900	130
Oct. 2–3	" "	52,200	2,300	310	700	44,800	700	19,400	60

Although *H. septentrionalis* was relatively scarce, it formed a major portion of the zooplankton biomass since an individual adult is about 3 mm long. The estimated ratio of volume of a live adult of each species to one of *D. ashlandi* is: *C. scutifer* 1.5, *B. coregoni* 1.5, *D. longispina* 2.5, *D. pribilofensis* 4.0, and *H. septentrionalis* 12.0. Thus in terms of relative biomass in the North Arm on August 5–6, the species were ranked from lowest to highest: *D. longispina*, *B. coregoni*, *E. nevadensis*, *D. pribilofensis*, *D. ashlandi*, *H. septentrionalis*, and *C. scutifer* (Fig. 11). If the above ratios were applied to the counts at other times of the year, for example in Table 3, this relationship might be somewhat different.

The diel vertical distribution of the zooplankton on August 5–6 at Nine Mile Creek (Fig. 11) is the basic pattern found in Babine Lake in 1967 for adults and for late copepodid stages or late instars. *Daphnia longispina*, *H. gibberum* (not shown), *E. nevadensis*, and both *Diaptomus* species were found largely above 12 m throughout the diel period whereas *C. scutifer* was common through the entire water column sampled. None of these species showed marked vertical movements although *D. ashlandi* tended to settle somewhat after dark. Both *B. coregoni* and *H. septentrionalis* displayed pronounced diel vertical movements, and these were in opposite directions.

Bosmina coregoni occurred mainly above 6 m during the daylight hours, but generally moved away from the surface at night (Fig. 11 and 12). This nighttime descent was usually very marked, and the maximum depth generally increased through the summer and fall. In the North Arm the depth of greatest abundance at nighttime was 6–9 m in June and July, 9–17 m in August, 10–24 m in September, and 1–15 m in October (Fig. 12). In the main lake the behaviour of *B. coregoni* was similar, but the nighttime maximum depth was somewhat deeper: 9–15 m in late July and early August and 21 m in late August.

From midsummer into the fall, *H. septentrionalis* exhibited an extremely marked diel vertical movement, as in the North Arm (Fig. 13); it also displayed major seasonal changes in vertical distribution. This species first appeared in the zooplankton samples in both regions of the lake in mid-June as nauplii and early copepodid stages, and until at least late June it was in greatest abundance in the top 6 m of water both day and night. By late July the population of *H. septentrionalis* in the North Arm was composed of late copepodid stages and adults and was making a diel ascent from a midday depth of greatest abundance of 10–27 m to a nighttime depth of 1–10 m. The midday depth in the North Arm increased during the summer and in late summer and fall the greatest abundance was at 24–32 m. On comparable dates the depth of greatest abundance of *H. septentrionalis* in the main lake was slightly greater than in the North Arm.

There was no evidence that the diel vertical movements of *H. septentrionalis* and *B. coregoni* in 1967 were affected in a major way by temperature stratification.

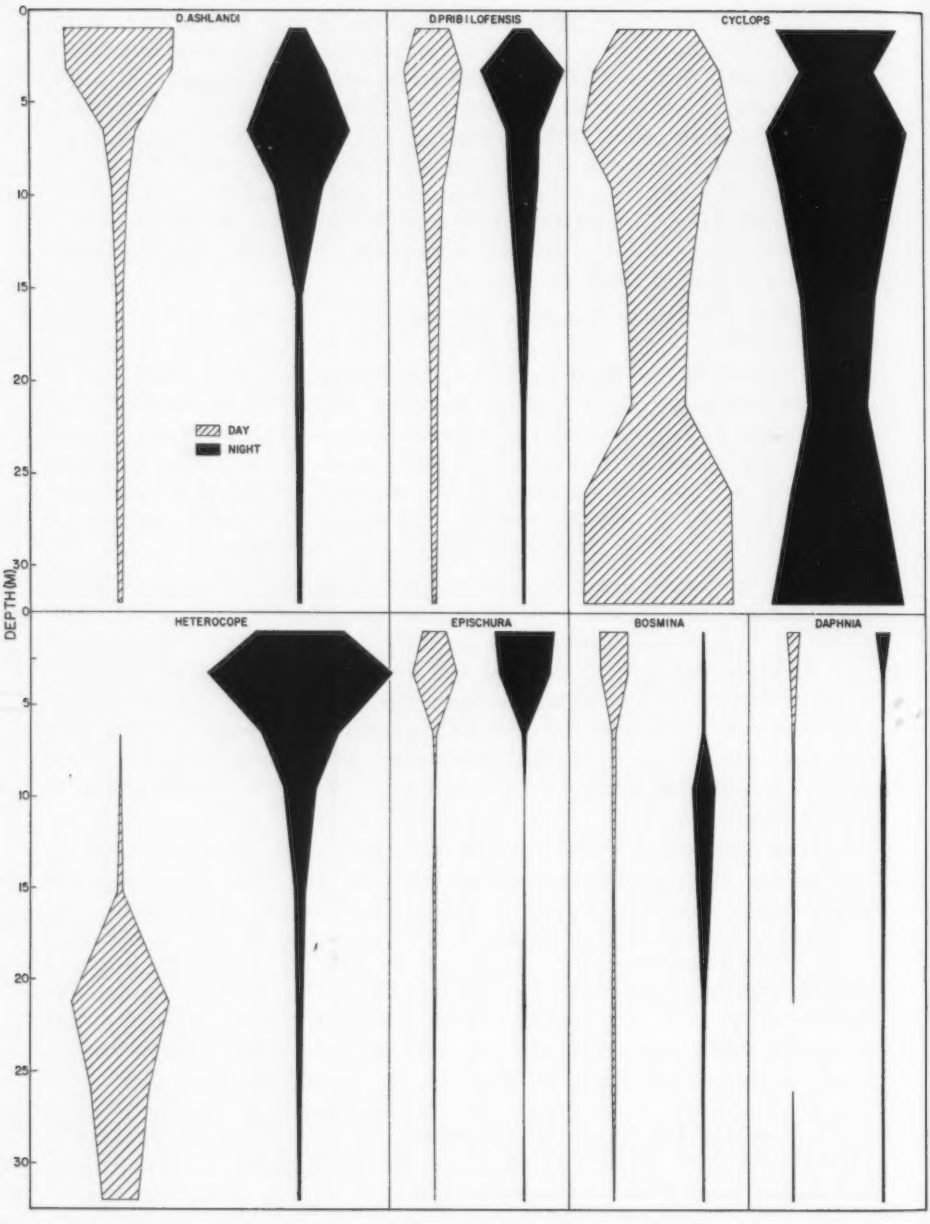


FIG. 11. Day and night average vertical profiles of the relative bulks of the major zooplankton species captured by Miller samplers near Nine Mile Creek on August 5–6. (The day profile is an average of 4 and the night of 2 series. See text for definition of relative bulk.)

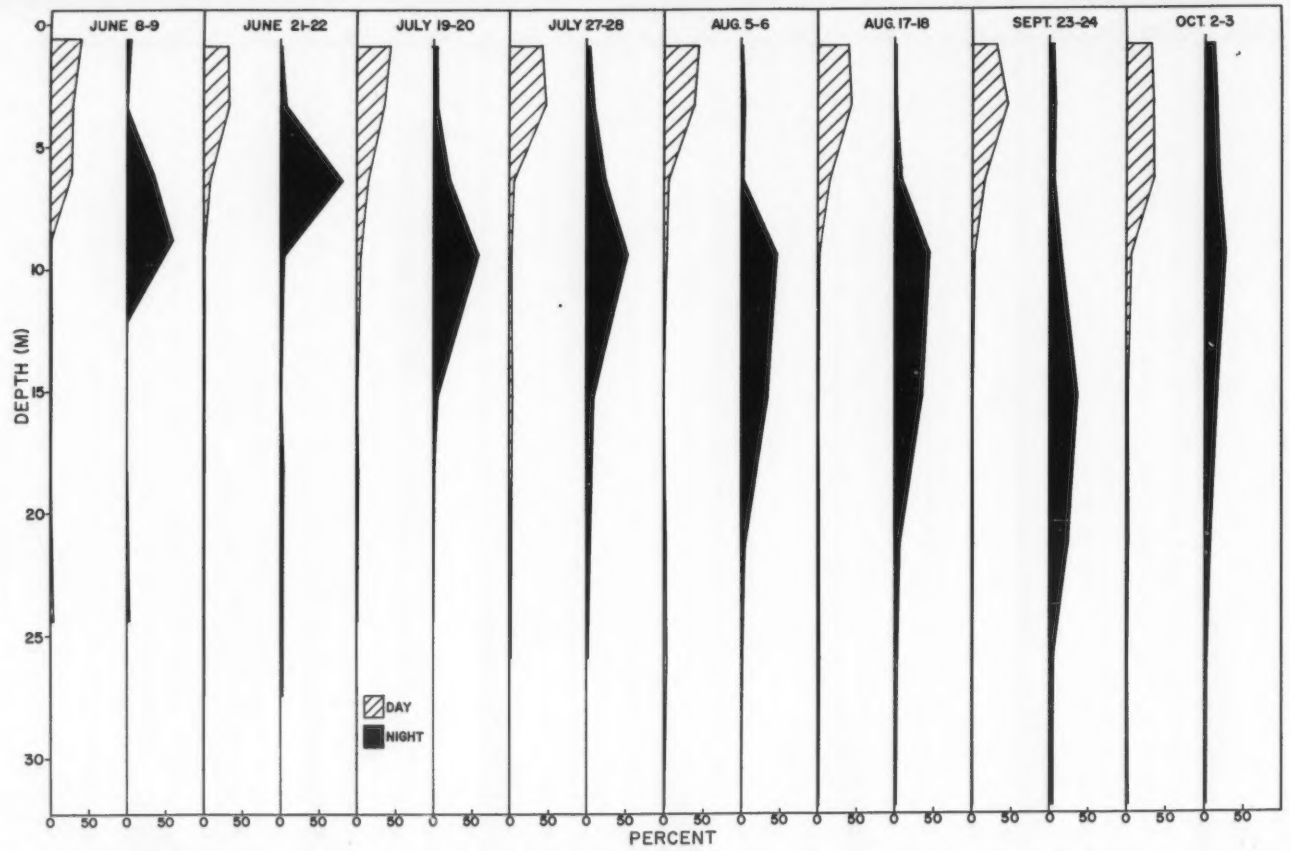


FIG. 12. Daytime and nighttime average vertical profiles of distribution (as percentages of total numbers) of *Bosmina coregoni* in the North Arm from June 8 to October 3.

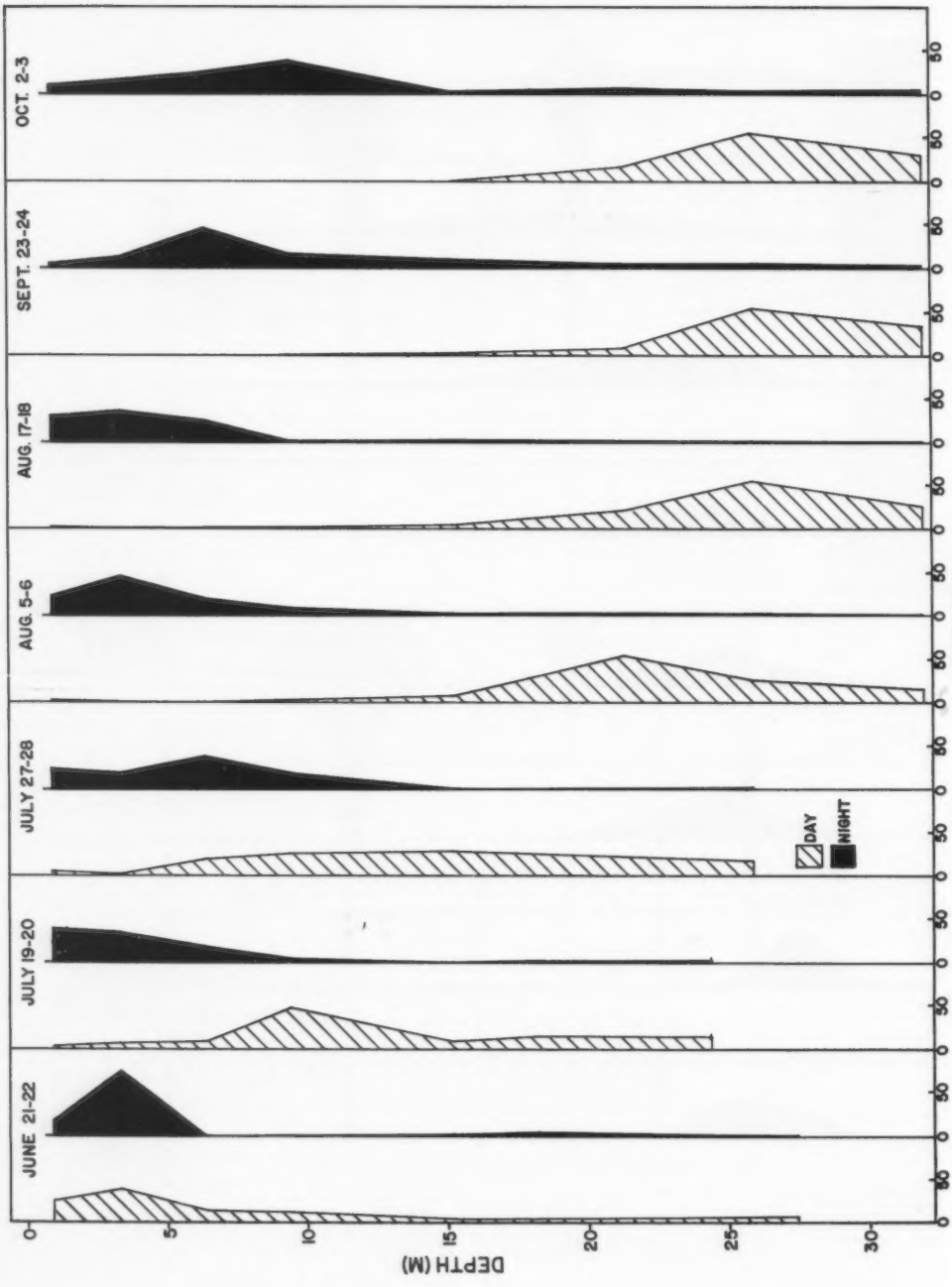


FIG. 13. Daytime and nighttime average vertical profiles of distribution (as percentages of total numbers) of *Heterocope septentrionalis* in the

DIEL FEEDING HABITS OF YOUNG SOCKEYE

By far the most abundant species in both percent composition and percent occurrence in the cardiac sections of stomachs of young sockeye taken in the Nine Mile Creek series were *H. septentrionalis*, *D. longispina*, and *B. coregoni* (Fig. 14). The total percent composition in all four series shows a major increase at dusk and a secondary increase in the morning representing two peaks of feeding. In the July, early August, and October series, the total percent composition at night (about 0200 hr) was low relative to that of the previous evening, whereas in the late August series there was little change between dusk and 0200 hr. The first three nights mentioned were relatively dark, either cloudy or moonless, or both, whereas the last was clear with a full moon; presumably the fish were feeding during the night of August 17–18. More daytime feeding occurred on July 27 than in the other series, and this feeding was almost entirely on *H. septentrionalis* (Fig. 14). The intensity of feeding apparently decreased markedly by October, when at the peak feeding period in the evening food comprised only about 23% of the volume of a potentially full stomach compared with nearly 40% earlier in the year. This decrease in feeding intensity in October was accompanied by changes in the diel movements of young sockeye as mentioned earlier.

Both the percent composition and percent occurrence of *H. septentrionalis* in the cardiac sections of stomachs during the day in July and August (Fig. 14) were usually higher in fish from the upper layer than in those from the lower layer. The importance of *H. septentrionalis* as a food item did not regularly increase in the evening. By contrast, the importance of *D. longispina* and *B. coregoni* as food items during the day was very low, whereas these species were generally most important at dusk and dawn. *Daphnia longispina* was particularly important in the late July–early August series, whereas *B. coregoni* was more important in late August and was most important in early October.

The diel feeding habits of young sockeye in early and late August in the main lake region (Fig. 15) were rather different from those for the North Arm. The total percent composition for both series at Pierre Creek shows a major increase at evening dusk, but only the August 28 series suggests early morning feeding. However, caution is necessary in interpreting part of the August 9–10 series since the 0800 and 1200 samples consisted of only 2 and 3 fish respectively. Although two layers of young sockeye usually occur in the main lake during the day (Fig. 9), samples from the upper layer were not obtained in either of these series because fish were scarce. The most common food items were insects (Fig. 15), mainly adult forms of Homoptera (especially Cicadellidae — leafhoppers) and Diptera (Chironomidae — gnats). *Daphnia longispina* was the most important food organism among the zooplankton, followed by *H. septentrionalis* and *B. coregoni*. *Daphnia longispina* was relatively common in the August zooplankton community of the main lake; the relative abundance (estimated in the same manner as for the North Arm in Table 3) was 4700 and 7900 on August 9 and 28 compared with the

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Fig. 13. Daytime and nighttime average vertical profiles of distribution (as percentages of total numbers) of *Heterocope septentrionalis* in the

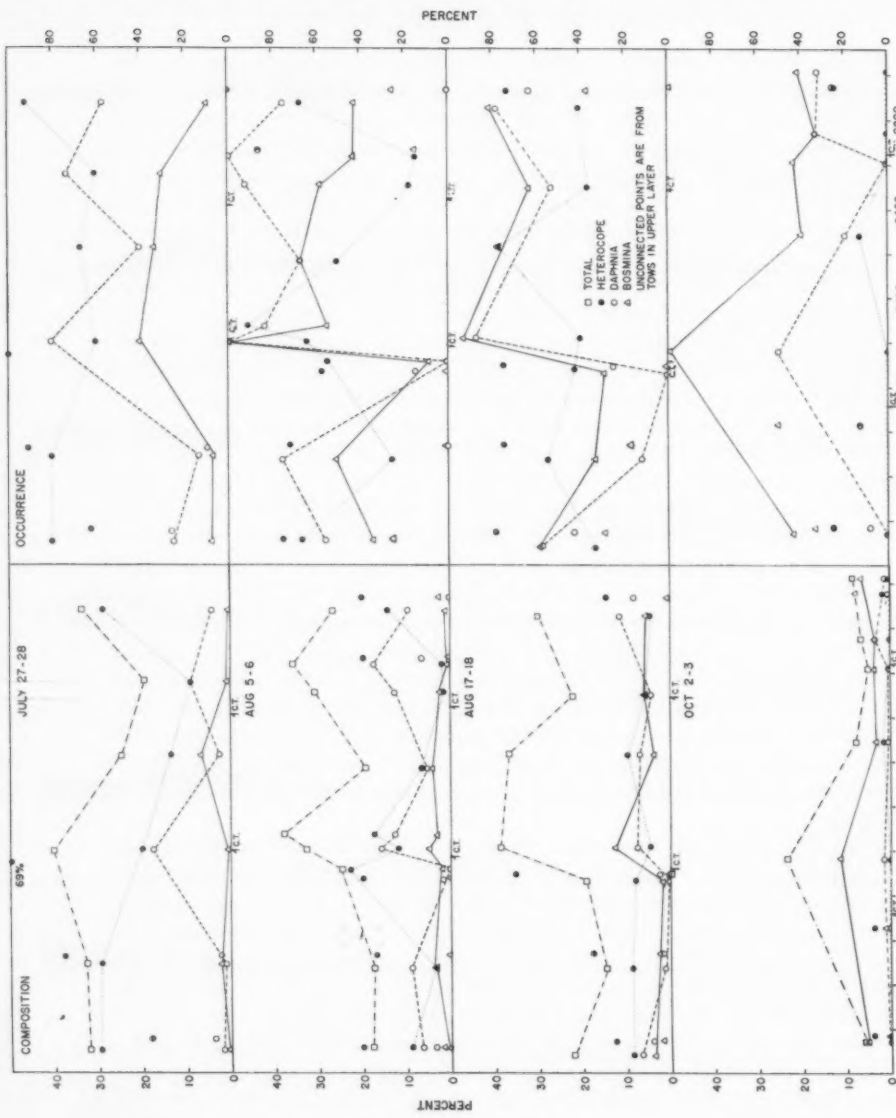

FIG. 14. Average diel percent compositions and percent occurrences of the most common zooplankton species in the cardiac sections of underyearling sockeye stomachs collected during four series near Nine Mile Creek. Average number of stomachs in each sample (time) is 15 (4–40). Time of civil twilight is indicated; time is Pacific Daylight Time. Differences (mainly in late

North Arm values of 1600 and 1400 on approximately the same dates. At the same time *H. septentrionalis* was much less common and *B. coregoni* more common in the main lake than in the North Arm.

A high degree of selective feeding on the zooplankton community by young sockeye is demonstrated by the "index of electivity" of Ivlev (1961,

FIG. 14. Average diel percent compositions and percent occurrences of the most common food items in the cardiac sections of underyearling sockeye stomachs collected during four series near Nine Mile Creek. Average number of stomachs in each sample (time) is 15 (4–40). Time of civil twilight is indicated; time is Pacific Daylight Time. Differences (mainly in late

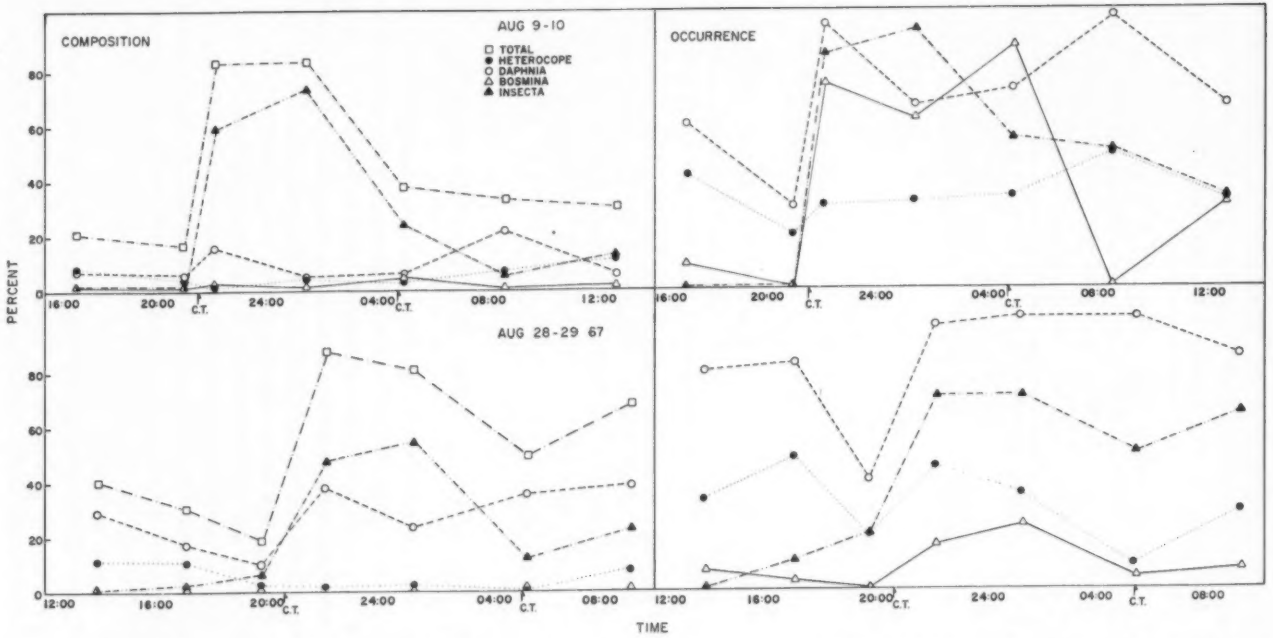

FIG. 15. Average diel percent compositions and percent occurrences of the most common food items in the cardiac sections of underyearling sockeye stomachs collected during two series near Pierre Creek. Average number of stomachs in each sample (time) is 23 (2–43). Time of civil twilight is indicated; time is Pacific Daylight Time.

p. 44–45). High values of this index were found for *D. longispina* in the evening and the morning on August 5–6, 1967 (Table 4). Presumably the high index values for *D. longispina* in both layers after the fish had descended (0615 and 0630 hr) reflected the intense feeding that occurred on that species at the surface at dawn since there were virtually no *D. longispina* below 10 m (Fig. 11). *Bosmina coregoni* was also high in the evening and at dawn, although not after the fish had descended. In the morning, after descent, *H. septentrionalis* was selected by fish in the upper layer where *H. septentrionalis* is relatively abundant but not the lower layer.

TABLE 4. A summary of Ivlev's (1961) index of electivity for underyearling sockeye feeding on the zooplankton community near Nine Mile Creek on August 5–6. Ivlev's index ranges from −1.0 to +1.0, where −1.0 denotes a prey species that is not utilized; .0, one that occurs in the predator's stomach at the same rate as it does in the community; and ≃+1.0, one that occurs in the stomachs but is extremely scarce in the prey community. The zooplankton were taken at 2000 hr for the 2200 hr sample of sockeye and at 0400 hr for the other three sockeye samples. The trawl at 35 m was sampling fish from the lower layer and the trawl at 20 m was sampling the upper layer.

Species	Purse seine at 2200 hr (n=11)	Purse seine at 0500 hr (n=12)	Trawl at 35 m 0615 hr (n=7)	Trawl at 20 m 0630 hr (n=7)
Diaptomus ashlandi	−1.0	−.9	−1.0	−1.0
Diaptomus pribilofensis	−.8	−.6	−.9	−1.0
Cyclops	−1.0	−1.0	−1.0	−1.0
Epischura	−.9	−1.0	−.8	−1.0
Heterocope	+.2	−.7	−.7	+.4
Bosmina	+.7	+.6	−.3	−.4
Daphnia	+.9	+.9	+.9	+.8

DIEL VERTICAL MOVEMENTS OF YOUNG SOCKEYE IN RELATION TO FEEDING

The existence of a double layer of young sockeye during midday in the North Arm from late July to early October has been documented in this paper. Both layers were about 6–9 m thick, and they were 9–12 m apart. The upper layer often appeared to be less dense than the deeper layer, and both layers tended to increase in depth through the season (Table 1). The separation between the two layers was essentially complete with the exception of October 2–3 (Fig. 4–8). I believe that an analogous double layering also occurs in the main lake, although it is not as marked as in the North Arm, perhaps because the fish population is much less dense. A double layer was evident in the daylight echo-sounder traces at Pierre Creek on August 9–10 and August 28–29 (Fig. 9). Unfortunately, no samples from the upper layer were obtained in the main lake in 1967.

In most of the 1967 diel studies, including the main lake, the daytime depth of the upper layer of fish indicated by the echo-sounder traces corresponded closely with the daytime depth range of *H. septentrionalis* (Table 1). Only

the July series in Table 1 failed to show such a correspondence, and this occurred when the *H. septentrionalis* population, composed mainly of copepodids, was relatively near the surface during the day (Fig. 13). The midday diet of young sockeye during the summer in the North Arm consisted mainly of *H. septentrionalis* with fish in the upper layer generally having higher percent composition and percent occurrence in the cardiac sections of the stomachs than fish in the lower layer (Fig. 14). A further indication that the upper layer fish are feeding more intensively than those in the lower is that the percentage of cardiac stomach sections containing organisms designated as fresh is much higher and those containing organisms designated as well digested is much lower in fish from the upper layer than in those from the lower layer. The data used are for 1200–1600 hr July 27–August 18 in the North Arm:

		Degree of digestion in %		
	No. of fish	Fresh	Moderate	Well
Upper layer	44	68	20	12
Lower layer	90	24	25	51

In July and August the *total* percent composition of food in the pyloric sections of stomachs from young sockeye (the squares in Fig. 16) taken from the upper layer until midday (approximately 0900 and 1300 hr) was consistently less than for fish from the lower layer. This suggests that those fish in the upper layer, in association with *H. septentrionalis*, had fed less intensively prior to descent (i.e., at dawn) than had the fish in the lower layer where relatively little zooplankton was available and the light intensity may be too low for effective feeding. By late afternoon (1600–2000 hr) in the July and August series, fish in the upper layer had pyloric sections with total food content as high as or higher than did those fish in the lower layer (Fig. 16). Fish in the two layers appear to completely mix near the surface each night, but whether the same individuals return to each layer day after day is unknown. Perhaps a new situation is developed each day in which some fish are attracted to or remain associated with *H. septentrionalis*, thus forming the upper layer, whereas the remaining fish (having fed to satiation?) commence their descent earlier, thus forming the lower layer. I suggest that this midday double layer of young sockeye during the summer in the lake is the result of a conflict between the "preferred" light intensity (the lower layer) and the food resource represented by *H. septentrionalis* (the upper layer).

Such a hypothesis on the formation of the two midday layers is considered in more detail by examining the August 5–6 series (Fig. 17). The total percent composition was highest in the evening and morning when the fish were at the surface or just after they had left. The total percent composition was less in the upper layer than in the lower in the morning, whereas it was about as great in the upper layer as in the lower in the evening. This suggests again that fish are in the upper layer feeding during the day because they did not obtain a sufficient ration when they were near the surface at dawn. The

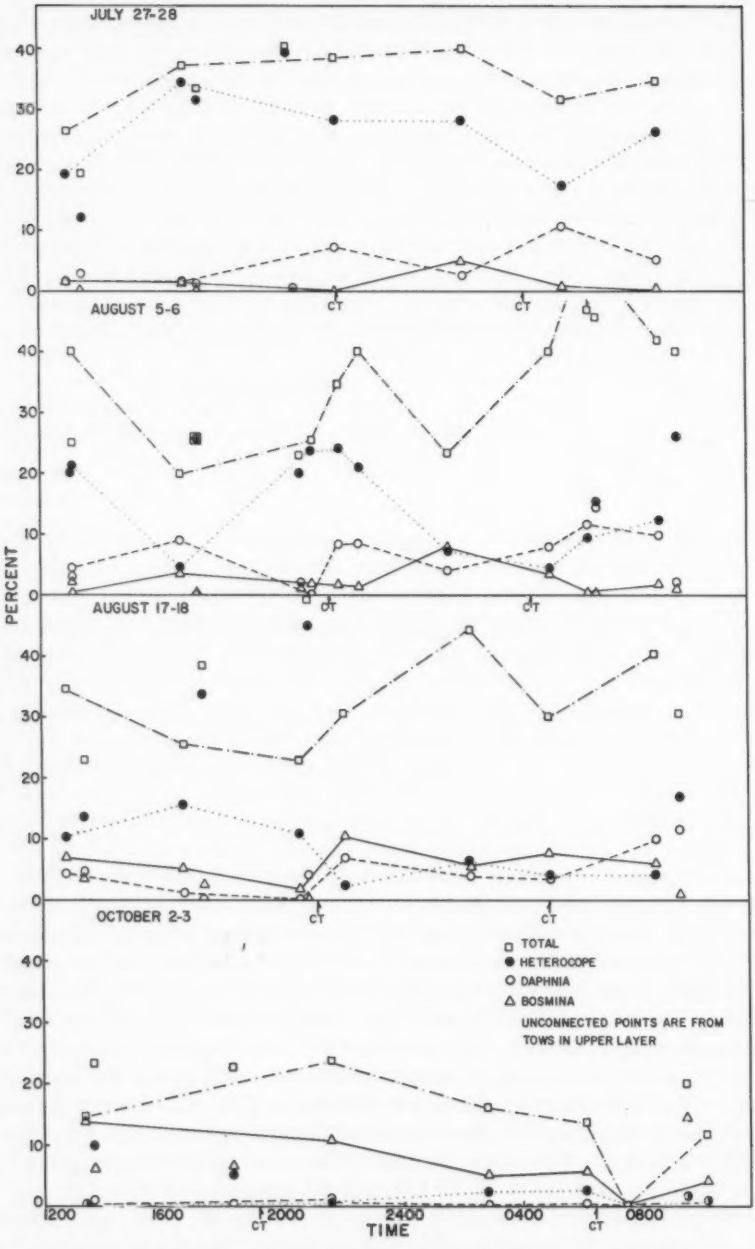

FIG. 16. Average diel percent compositions of the most common zooplankton species in the pyloric sections of stomachs from underyearling sockeye collected during four series at Nine Mile Creek. The average number of stomachs in each sample (time) is 15 (4–40). Time of civil twilight is indicated; time is Pacific Daylight Time.

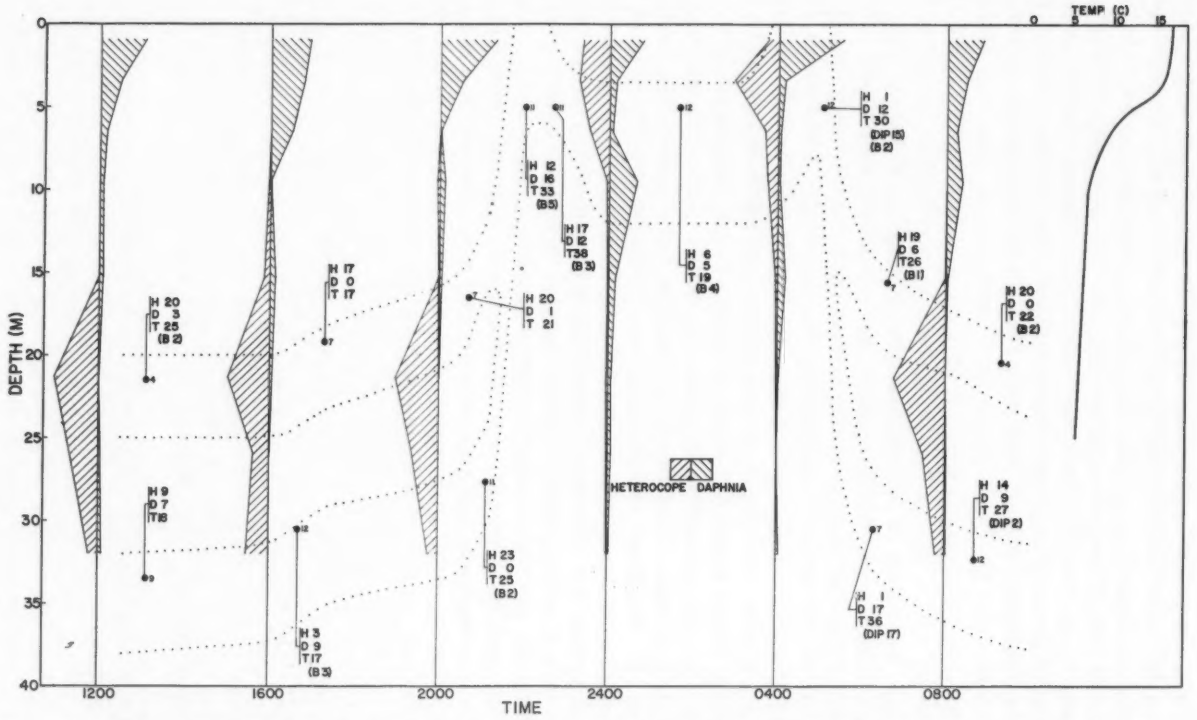

FIG. 17. Relationships at Nine Mile Creek on August 5–6 between the diel vertical distributions of underyearling sockeye (dotted lines), the diel vertical distributions of *Daphnia longispina* and *Heterocope septentrionalis* (hatched areas), and the average percent compositions of major food items in the cardiac sections of stomachs from underyearling sockeye captured by midwater trawl and purse seine at indicated times, depths, and sample size. Percent composition is indicated for *Heterocope* (H), *Daphnia* (D), *Bosmina* (B), Diptera (DIP), and total (T). Fish distribution is estimated from echo-sounder traces. Zooplankton distribution is relative (in percent) only for each species and time. Sunset was at 2055 hr, sunrise at 0515 hr, and civil twilight at 2140 and 0430 hr Pacific Daylight Time.

percent composition of *H. septentrionalis* in stomachs taken from fish in the upper layer was consistently higher than that in the lower layer, suggesting that this was the main daytime food item. In the evening and through to dawn the relative importance of *H. septentrionalis* diminished whereas that of *D. longispina* and *B. coregoni* increased (Fig. 17). Thus, during the most intensive periods of feeding (dusk and dawn), the young sockeye are apparently selecting the least common zooplankters (Fig. 11 and Table 3). Diptera were important at dawn and were later found only in fish in the lower layer.

DISCUSSION

This study, conducted in two different limnetic regions of Babine Lake in 1967, has revealed in general terms where the young sockeye are and what they are doing in relation to their potential food species at any time during a 24-hr period in the summer and fall. The fish undertake a diel vertical movement in which they pass from a midday depth of 18–40 m to the surface in the evening, disperse to a depth of 5–18 m after dark, and descend again in the morning. During the day the fish are in two layers centered at about 20 and 35 m. The young sockeye feed most intensively when they are near the surface in the evening and again in the morning. At this time they feed selectively on *D. longispina* and *B. coregoni*. *Heterocope septentrionalis* is utilized mainly during the day by fish in the upper layer. Fish in the lower layer feed at a very low intensity if at all. Of the eight major species of zooplankton only *H. septentrionalis* and *B. coregoni* undertake marked diel vertical movements and these are in opposite directions.

The precise timing of the diel vertical movements of young sockeye in Babine Lake in midsummer suggests strongly that the intensity of underwater illumination may play an important role. The evening ascent coincides with decreasing light until the fish all approach the lake surface (Fig. 4 and 5). Immediately after this the fish disperse throughout the top 5–18 m where they remain until first light of morning. This dispersion after dark is probably because the minimum intensity of illumination required for schooling is no longer available — presumably the fish are not able to orient to each other. Blaxter and Parrish (1965) found that herring schools dispersed at light intensity below 10^{-1} lux. The threshold for the cessation of feeding (probably approximating that of schooling) is about 10^{-3} lux ($\approx 10^{-4}$ ft-c) for sockeye fingerlings (Brett and Ali, 1958) although feeding intensity began to decrease at 1 lux.

Numerous studies suggest that diel vertical movements of fish are associated with major morning and evening changes in light intensity as reviewed by Woodhead (1966, p. 362–370). However, few investigators measured underwater light intensity in these studies. Blaxter and Parrish (1965), studying the diel vertical movements of herring, showed a movement that was roughly related to changing light intensity, but they could not establish that the depth of herring at midday depended on the absolute light intensity. The

diel vertical distribution of peamouth, *Mylocheilus caurinus*, in a small lake appeared to be related to light penetration; fish movements roughly corresponded to the depth of the 1 lux isophot (Northcote et al., 1964).

Of the zooplankton species of the lake, only *H. septentrionalis* and *B. coregoni* undertook major diel vertical movements (Fig. 11–13); these are rather precisely timed and are probably also related to changes in the intensity of underwater illumination. Such a relation has been indicated for other invertebrates including *Chaoborus* (Teraguchi and Northcote, 1966) and *Daphnia* (McNaught and Hasler, 1964; Ringelberg, 1964). As was revealed earlier, *H. septentrionalis* undertakes a typical nocturnal vertical migration whereas *B. coregoni* undertakes a reversed vertical migration in the terminology of Hutchinson (1967). The reversed movement of *B. coregoni* in Babine Lake (near the surface during the day and at depth at night) is contrary to the diel activity of most species of zooplankton reported from other lakes (Hutchinson, 1967). However, Hutchinson (p. 740–742) relates several rather clear examples of this type of diel movement by zooplankters, none of which is in the genus *Bosmina*.

The hypothesis offered earlier to the two daytime layers of young sockeye was based on the best available data. One alternative explanation might be that these two markedly different behaviour patterns are stock specific, but since I have recorded this phenomenon throughout the North Arm and as far south as Pierre Creek in the main lake, this is doubtful. (No midday echo soundings were made farther south in either 1966 or 1967.) The fish comprising the two layers in the main part of the lake could be the progeny of at least five major and distinct spawning stocks: Babine River above and below Nilkitkwa Lake, Morrison River, Fulton River, and Pierre Creek (Fig. 1). Another explanation might be that one layer is comprised of underyearling kokanee, and this cannot be dismissed with the information at hand.

Presumably the diel vertical movements of young sockeye in the lake are a manifestation of natural selection, but exactly what the selective pressures are (or were) is unknown. There may be a physiological advantage for an animal to feed in the warm surface waters for a short time and to spend the remaining time digesting food in the cool hypolimnion as suggested by McLaren (1963). The vertical movement takes sockeye from the hypolimnion at temperatures of 5–7 C to the epilimnion at temperatures of 14–19 C. The summer thermocline in the North Arm usually occurs at 3–7 m (Fig. 10). At night the majority of fish are below the thermocline. Such a behaviour may also be in response to a predator. It seems that a fish that spends most of its life in twilight would be less likely to be preyed upon than would the same fish found in higher intensity of illumination. However, there seem to be few predators of underyearling sockeye in the limnetic zone. For example, 39 night purse-seine sets made in the North Arm when sockeye were within 15 m of the surface resulted in about .5 rainbow trout per set and lake trout (*Salvelinus namaycush*) were even scarcer.

When the diel activity of young sockeye in the limnetic zone of other nursery lakes is studied, I expect that the same essential elements of a vertical movement will be present that are described here for Babine Lake. In most of the Alaska lakes, the young sockeye are available to two-boat, surface townets of various sizes only during periods of dusk and darkness. In the Wood River Lakes, Alaska, echo sounders of relatively low frequency indicate young sockeye are not present near the surface during midday but are abundant there at night (Pella, 1968). With the same echo sounder similar behaviour of young sockeye is suggested in Iliamna Lake, Alaska (Dr R. L. Burgner, personal communication). Unfortunately, these low frequency instruments are usually not capable of detecting young sockeye to depths as great as can the 175 kHz echo sounder used at Babine. A major modification in the diel vertical movements appears to occur in Lake Washington near Seattle, where underyearling sockeye rarely ascend higher than 15 m below the surface (Mr J. C. Woodey, personal communication). This might be common in lakes at the southern end of the sockeye salmon's range, where epilimnion temperatures are frequently above 20 C in midsummer.

Clarity of the lake water probably has a profound effect on the amplitude of the diel movements. Recently (September 1969), using the same 175 kHz sounder, I found that young sockeye in Great Central Lake, Vancouver Island, B.C., are in schools at 55–79 m at midday with a maximum density at about 70 m; they approach the lake surface at evening dusk and are 10–20 m below the surface after dark. Target identification was substantiated by midwater trawling. This lake is more transparent than Babine Lake with a September Secchi depth of 14 m compared with about 4 m.

Where the lake is extremely turbid from glacial runoff, the amplitude of the vertical movements may be greatly reduced; young sockeye are sometimes captured by midday surface tows in Iliak Arm of Naknek Lake, Alaska (Dr C. J. CiCostanzo, personal communication) and in Owikeno Lake, B.C. (Ruggles, 1965). I found in Black Lake, a lake in the Chignik River system, Alaska, with a maximum depth of 6 m, that young sockeye were not normally captured during the day by surface townets but were abundant there at night (Narver, MS, 1966). However, on dark days or on days when wind-generated waves had disturbed the bottom sediments to make the water highly turbid, young sockeye were captured by surface townet.

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Marking Animals with Micro-Tags of Chemical Elements for Identification by X-Ray Spectroscopy

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This report describes a method of marking living organisms. As an illustration of the method, foreign chemical elements are incorporated into small salmon and the analytical technique of X-ray fluorescence spectroscopy is used to detect and measure minute quantities in the tissues. Mixtures of elements, either in equal or varying concentration, are used to generate a code. Decoding is readily accomplished by irradiating the tagged organisms and monitoring the X-ray fluorescence produced. A large number of unique combinations appear possible using the method as described. Mixtures of chemical elements (atomic numbers 37–69) were suspended in silicone rubber and injected directly into fish tissue. In this manner tags 1 mm^2 in area were incorporated in the tissue and decoded by nondestructive analysis.

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INTRODUCTION

IN BIOLOGICAL STUDIES involving many seemingly identical organisms it is frequently necessary to distinguish one organism or group of organisms from another. In the past, marking of specimens has been accomplished by a wide variety of techniques but for small organisms current marking practices are generally limited in one way or another. We discuss here a number of possible marking methods and describe in detail a new method that has been successfully used to mark fish ranging in length from 3 to 5 cm with many different tags, and that allows for rapid and repeated identification of live specimens. This marking method is very general and most likely could be used to tag a wide variety of small organisms.

To mark the fish a small tag consisting of a mixture of chemical elemetns is implanted in the animal just under the skin. The number of different tags attainable is just the number of unique combinations of elements that can be

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implanted and detected. Detection of the elements present in the tag is accomplished without removing it from the organism by the method of X-ray fluorescence spectroscopy. This method of chemical analysis has been used for several years but recent advances in semiconductor X-ray detectors have made the technique much easier and more rapid than was possible earlier (Bowman et al., 1966). To perform the chemical analysis one irradiates the tag with gamma rays from an external radioactive source. Upon striking the sample the gamma rays eject the innermost electrons of the elements in the tag. When another electron of the atom falls into the vacant orbit, X-ray radiation is emitted. Many of the X-rays leave the fish and are detected with a Si(Li) semiconductor X-ray detector. The energy of these X-rays depends upon the element from which they are emitted. Using a detector of sufficient energy resolution, one can determine which elements are present in the tag.

DETECTING FOREIGN ELEMENTS IN TISSUE

We considered several methods of detecting mixtures of foreign material in tissues. In general, tags could be made up of mixtures of radioactive isotopes, mixtures of fluorescent compounds, and mixtures of chemical elements that are normally not radioactive or fluorescent.

Mixtures of radioactive isotopes can be readily resolved with a suitable radiation detector. The detection and separation of mixtures of fluorescent compounds may be possible using a spectrophotometer. There are a number of practical methods of detecting and separating mixtures of chemical elements. Neutron activation is one such method. In this technique a sample is bombarded with neutrons that are captured by the nuclei of the elements in the mixture and become radioactive. Identification is easily accomplished, since the radiation given off is characteristic of each isotope. Identification of the components of mixtures is also possible by nuclear magnetic resonance analysis. In this method the sample is placed within a static magnetic field and actived by a radio-frequency electromagnetic field. The frequency of the electromagnetic field necessary to reverse the nuclear polarization is characteristic of the isotope (Andrews, 1956). A third method employs the use of lasers. In one application, samples of chemical elements are evaporated by exposure to the laser and the resultant light emission spectrum is then analyzed (Margoshes, 1968). The final method considered makes use of X-ray fluorescence spectroscopy and is the one chosen for the tagging method described here.

These detection schemes were evaluated as follows: (1) Would the organism be adversely affected in the process of reading the code? (2) Could the tag be read if the organism moved slightly with respect to the detector system? (3) What is the physical form of the tag for each detection method? (4) What effect would there be on other organisms that ingest the tagged animals, and what would be the hazards to public health if the tagged organisms are liberated? In evaluating these questions, we concluded that each of the above techniques has unique advantages and disadvantages. It was concluded that

X-ray fluorescence spectroscopy is suitable for population studies on small salmonids.

EXPERIMENTAL METHODS

Preparation and Implantation of the Tag

The tags are prepared by mixing finely powdered oxides of the desired chemical elements in a carrier of silicone rubber.[1] A catalyst is added and the mixture is injected through a fine needle a distance of approximately 1 mm into the fish. The catalyst causes the rubber to harden so that the chemical elements are suspended in a permanent innocuous form.

Suspensions with concentrations no greater than 0.6 g of screened oxides per 1 cc of elastomer were used because more concentrated mixtures were difficult to inject through the 25-gauge needle. At this concentration the elastomer also shows no apparent change in properties.

The sensitivity of the X-ray fluorescence system dictates the smallest amount of each element that may be detected and thus determines the smallest size of the tag. With the sensitivity of the present system and with the concentration quoted above, it is easy to detect mixtures of three elements in a tag of total mass 0.3 ± 0.2 mg. The hardened tags were observed to be fusiform in shape with length and diameter of 1.8 ± 1.0 mm and 0.9 ± 0.6 mm, respectively.

Elements with atomic numbers 37–64 (excluding 43, 51, 52) were chosen to make up the tags. These particular elements were chosen because they are not normally abundant in fish tissues and because their X-rays are sufficiently energetic to penetrate through the fish tissue to the detector. There are therefore 24 elements to choose from to make tags. The following 12 elements were chosen from the above series to test the method: samarium ($Z = 62$), neodymium ($Z = 60$), praseodymium ($Z = 59$), cerium ($Z = 58$), lanthanum ($Z = 57$), barium ($Z = 56$), cesium ($Z = 55$), tin ($Z = 50$), indium ($Z = 49$), silver ($Z = 47$), molybdenum ($Z = 42$), and strontium ($Z = 38$). With 3 elements or less chosen out of a group of 12 there are 298 unique combinations. This number of combinations was adequate for a planned experiment, in which 166 different groups of fish required marking.

Fish as small as 2.8 cm in length have been marked with the tags described above. The tagged fish in this study were 15-month-old sockeye salmon (mean size 6.6 cm, range 5.2–8.3 cm). At the onset of an experiment designed to study the long-term effects of these tags on fish, several tagged fish from each group were preserved in formalin.

Detection of Elements by X-Ray Fluorescence

The X-ray fluorescence system used for chemical analysis of the tag was patterned after that initially developed by Bowman et al. (1966) and further refined by Giauque (1968). For this discussion it is convenient to consider the system to consist of the following four parts (see Fig. 1): (1) the radioactive source and target assembly; (2) the liquid nitrogen-cooled X-ray detector and preamplifier and vacuum envelope; (3) the pulse amplifier, the detector bias power supply, and the pulse generator; and (4) the pulse height analyzer and teletype output. Since all of the parts are either commercially available or have been described in detail elsewhere, we point out only the important details.

The Radioactive Source and Target Assembly

The primary gamma rays were the 59.5 keV gamma rays of a 20 mCi Americium-241 source. The Americium-241 was mounted in an annular groove on a lead block through which in addition was drilled a concentric $\frac{3}{8}$-inch diam hole. The fish to be analyzed is placed at the opposite side of the source block and is thereby shielded from the Americium radiation. Mounted on an additional lead block is an annular thulium target that is concentric with a $\frac{1}{4}$-inch diam hole. The source and target holders are sandwiched together and placed near the X-ray detector.

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[1]Silastic No. 384 Elastomer. Dow Corning Medical Products Division, Midland, Michigan, USA.

The assembly is designed so that the ^{241}Am radiation is able to strike neither the X-ray detector nor the fish but must instead strike the thulium. The ^{241}Am gamma rays that irradiate the thulium target cause thulium K X-rays to be emitted that, because of the hole in the source block, are able to irradiate the fish. The fluorescent X-rays from the fish then pass through the holes in the source and target blocks to the detector. Thulium was chosen for the target material because its 50 keV X-ray is closer in energy to the X-rays of the elements used in the tag than is the Americium gamma ray. The entire source-target assembly was borrowed from the Lawrence Radiation Laboratory at Berkeley and has been described by Giauque (1968).

X-RAY DETECTOR AND PREAMPLIFIER

Both the X-ray detector and preamplifier along with the associated cryogenic and vacuum hardware were purchased as a unit (Model XS-25-DSR) from Nuclear Diodes, Inc. through BRH Associates of Montreal. The detector is a 3 mm thick lithium drifted silicon detector with 40 mm^2 area (Model LS-310). The preamplifier is model 102 SN 69-3 operated with time constants of 6.4 μsec in both the first differentiator and integrator and 1 msec in the second differentiator. The resolution of the system for 6.4 keV X-rays is 345 eV and for 14.4 keV X-rays is 393 eV. To distinguish the X-rays of elements that are adjacent to each other in the periodic table the energy resolution of the detector-preamplifier system must be less than the difference of the energies of the X-rays of the elements. Because the X-rays of the elements chosen for the tags differed in energy by more than 1 keV, the energy resolution of this system was more than adequate.

To achieve the necessary energy resolution the detector and part of the preamplifier must be cooled with liquid nitrogen (T = 77 K). This cooling was achieved with a 30-liter liquid nitrogen dewar that required refilling every 2 weeks.

PULSE AMPLIFIER, DETECTOR BIAS POWER SUPPLY, AND PULSE GENERATOR

The pulses from the preamplifier were further amplified by a Tennelec model TC202 linear amplifier. This unit required the use of a TC15 transfer module. Both of the units were subsequently replaced by a TC203BLR amplifier that provided base line restoration.

The detector was operated with a bias voltage of 470 v from a Tennelec TC908 power supply.

A Tennelec TC812 pulse generator was used to test the electronics by injecting a pulse into the preamplifier to simulate an X-ray pulse. The pulse generator is also useful for calibrations.

All of the above Tennelec modules were powered by a Berkeley Nucleonics AP-21 power bin.

PULSE HEIGHT ANALYZER

After amplification by the TC203 linear amplifier the pulses are sent to an Intertechnique (SA-41) 400 channel pulse height analyzer. In practice only 100 channels are necessary to accumulate and display the X-ray spectrum of a tag but the additional 3 groups of 100 channels are useful for overlays to compare one spectrum to another. The analyzer was obtained with a model C40 amplitude coding/module (analogue to digital converter) and a H23 timing module.

The analyzer sorts the incoming pulses according to the amplitude of the pulse (i.e., the energy of the X-ray) and stores a count in the channel corresponding to the amplitude of the pulse — the bigger the amplitude of the pulse, the higher the channel number the count is stored in. After accumulating many pulses the information may be displayed on the oscilloscope that shows the number of counts versus channel number. However, for more detailed analysis the number of counts in each channel is punched out on paper tape with a teletype (ASR33) unit. The paper tape is then fed into a computer for subsequent analysis.

RESULTS OF TAGGING

The samples were placed as close to the source-target assembly as physically possible and exposed to radiation (Fig. 1). Gain settings on the amplifier and pulse height analyzer were adjusted to allow for the detection of elements

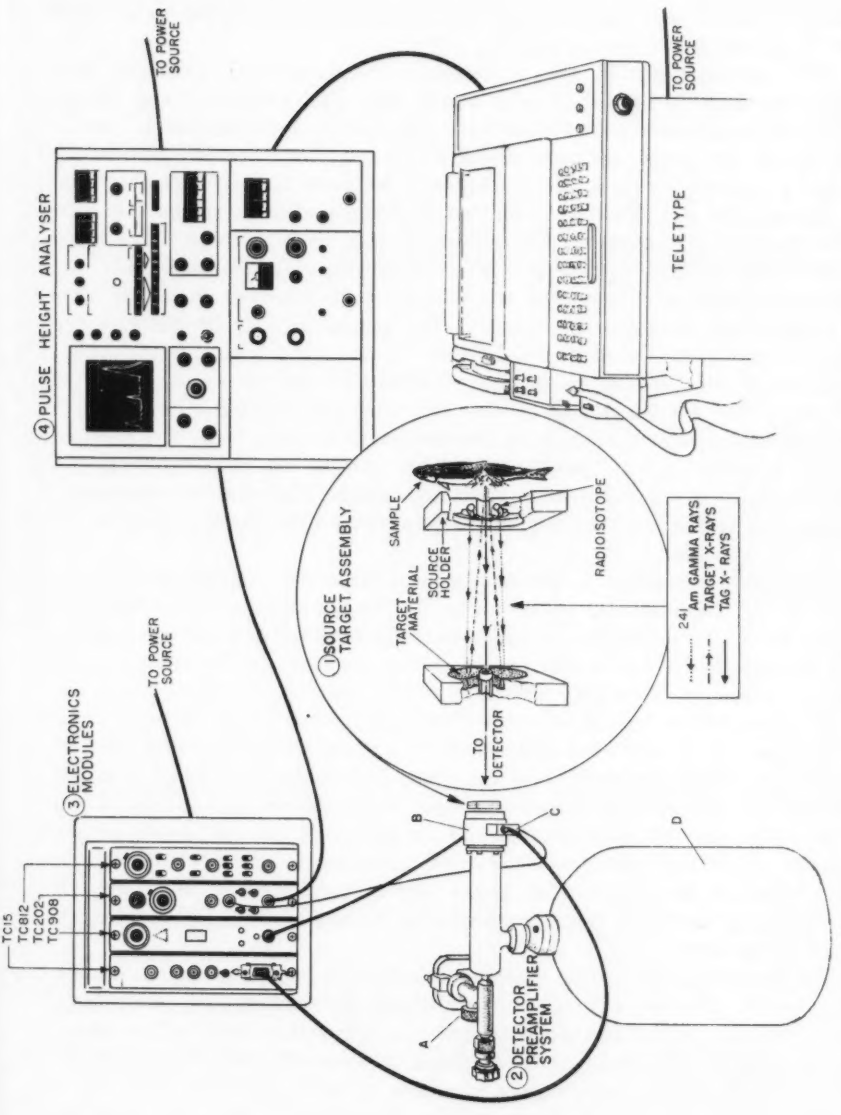

FIG. 1. X-ray fluorescence tag decoding system. The source-target assembly has been expanded to illustrate the X-ray paths. In practice the source and target holders are sandwiched together and are placed near the detector housing at B. The light cable attached to the preamplifier at C carries the X-ray pulses to the amplifier (TC202). The amplified signals are then sent to the pulse height analyzer for amplitude analysis. The vacuum pump is designated by A and the 30-liter liquid nitrogen dewar by D.

with atomic numbers 37 through 69. The pulse height analyzer was adjusted so that only 100 of the 400 channels were used to accumulate data for a pre-set time (clock) interval. At the end of the time interval, the number of counts recorded in each of 100 channels were printed on the teletype and simultaneously punched on paper tape. The data were then compressed into a 50-word array

by pooling the counts from two adjacent channels, and graphed on an IBM 1130 computer and Calcomp plotter.

The spectrographs in Fig. 2 illustrate the fluorescence patterns from samples exposed for 30 sec, 3 min, and 8 min. The spectra in Fig. 2*a* are typical of an unmarked freshwater fish. The two pronounced peaks, one on each side of the graph, are background peaks and have been included in the window as reference points. The background peak on the left originates from the fluorescence of L X-rays of the lead shielding and the peak on the right is due to back-scattering of the thulium X-ray by the sample. The three figures of the second row (Fig. 2*b*) are from fish injected with only the elastomer compound. Note that the silicone rubber carrier does not alter the background spectrum significantly. The graphs in Fig. 2*c* illustrate the change in the spectrum when the tag has the rare earth element lanthanum mixed into it. In this case the peak at channel 63 is due to the K_α X-ray of lanthanum. The graphs in Fig. 2*d* were generated by exposing a large sample of lanthanum–elastomer mixture in the absence of the fish. Here it is possible not only to detect the K_α peak (channel 63) but the K_β peak (channel 78) as well. Peaks in an unknown sample are readily identified by comparing the channel numbers of the unknown X-ray peaks to channel numbers of known X-ray peaks.

Noticeable variations in the number of counts per channel exist in the graphs in the columns and rows of Fig. 2. The variations from column to column are due to differences in count time. Increasing the counting interval tends to smooth the curves and reduces the uncertainty in the identification of peaks. The same effect may be had by increasing the strength of the radioactive source. Other factors can also affect the spectra as can be seen from the variations of spectra from row to row (i.e., the spectra of samples exposed for the same period of time). These variations are due to the kind of elements in the sample, the distance from the sample to the source, the position of the sample within the field of ionization, and the size of the sample. These factors affect the count rate and hence the time necessary to identify the tag. In many instances, identification of peaks was accomplished in less than 15 sec. With optimization of the above variables it should be possible to identify the tag in less time.

The spectrographs in Fig. 3 were generated by exposing tagged fish for 3-min periods. The vertical lines drawn through the background peaks form ready reference points and demonstrate the consistency with which these peaks appear in the graphs. Between these background peaks lie the X-ray peaks of 32 elements, combinations of which in principle could make up a tag. The graphs in Fig. 3 illustrate the X-ray peaks from 12 elements distributed between the background peaks. The heights of the X-ray peaks compared with the heights of the background peaks vary considerably on the graphs. The ratio of these heights was found to be largely governed by the size of tag, the depth of the tag in the fish, and the orientation of the fish with respect to the source. One can optimize these variables by using

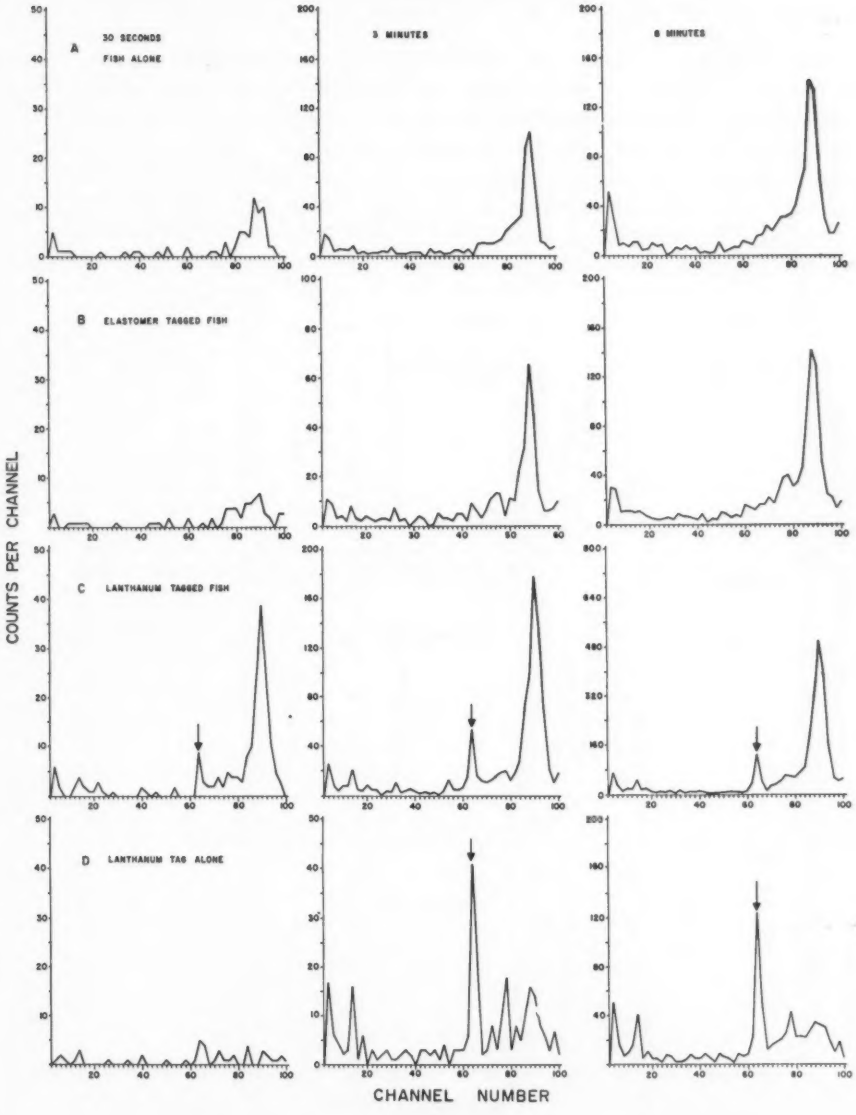

FIG. 2. Spectrographs of samples exposed at different time intervals: (A) the spectrum from an untagged fish; (B) spectrum from a fish injected with elastomer only; (C) spectra of fish tagged with lanthanum added to the elastomer; (D) spectra of elastomer lanthanum mixture (the tag material without fish). The arrows indicate the K_α X-ray peaks. Note the vertical scale is not the same for all graphs.

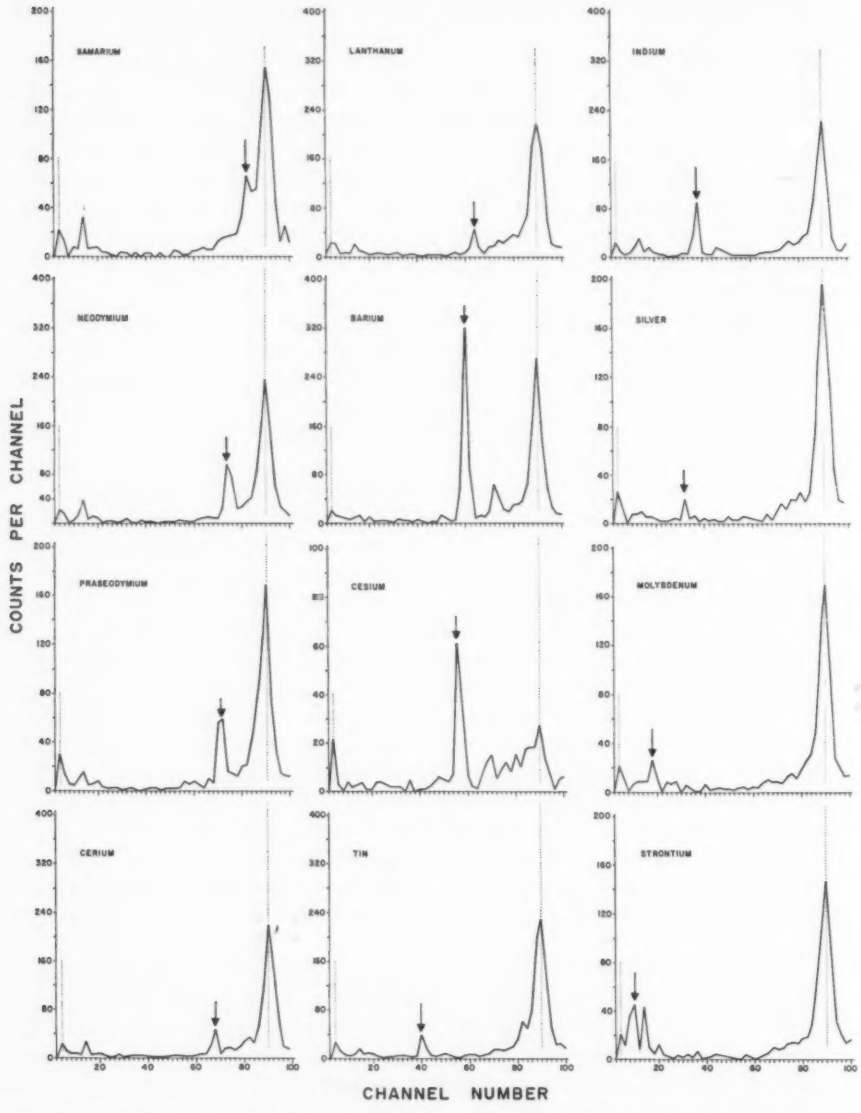

FIG. 3. Spectrographs of fish tagged with the oxides of 12 different elements suspended in silicone rubber. The dotted lines on the left of the graph indicate the background X-ray peaks from the lead shielding. Dotted lines on the right indicate background peaks generated by back-scatter from the fish. All samples were exposed for 3 min. Arrows indicate the K_α peaks of the elements.

as large a tag as possible, by injecting the tag just under the skin and in the same area of the fish, and by improving the "source-target assembly."

Spectrographs from fish tagged with all possible combinations of the elements neodymium, praseodymium, and cerium are shown in Fig. 4. The weights of the elements used to make the mixture were adjusted to give peaks of the same height. The graphs illustrate the ease with which combinations of three elements adjacent to each other in the periodic table may be detected within the fish. The ability to detect adjacent elements in this "window" illustrates that a large number of unique tags are possible. The number of distinct combinations and tags possible using n different elements is 2^n.

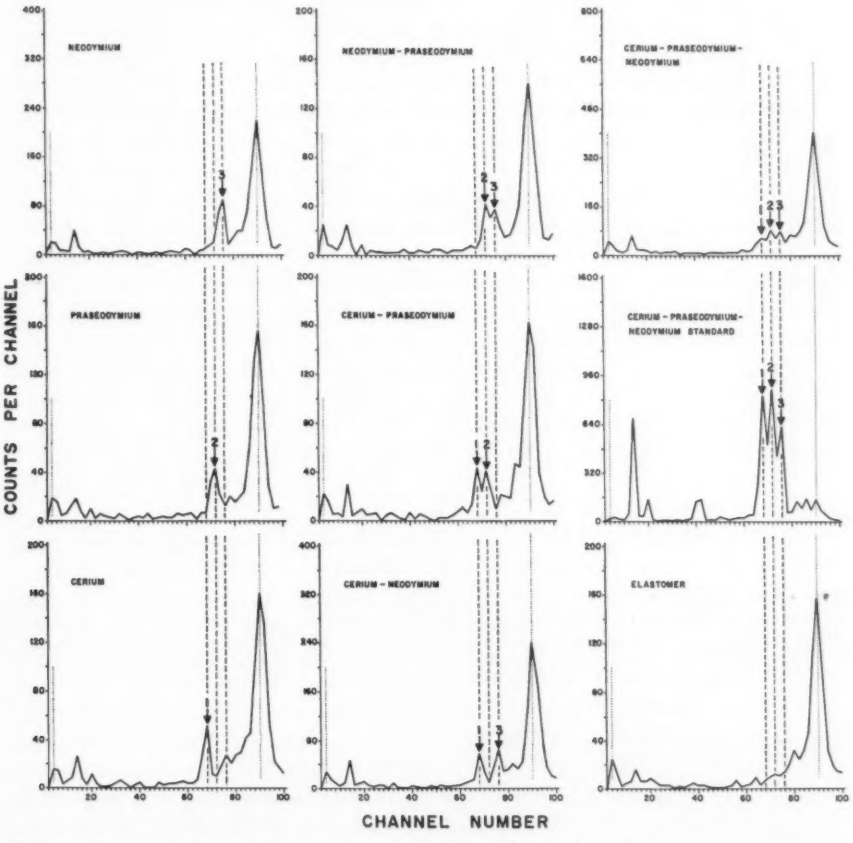

FIG. 4. Spectrographs of fish tagged with combinations of the elements neodymium, praseodymium, and cerium. Spectra of the elastomer tag containing all three elements, and an elastomer-injected fish are included. Samples were exposed for 3 min. The broken lines align channels where the K_α peaks of the elements would occur. The dotted lines connect the background peaks as in Fig. 3. Arrows indicate the K_α peaks of the elements: 1 — CeK_α, 2 — PrK_α, 3 — NdK_α.

If the number of concentrations of the different elements is larger than 2, the number of combinations that is possible is greater than 2^n. In fact, the number of combinations possible is k^n where k is the number of different classes, i.e., concentrations, and n is the number of different elements. The spectographs in Fig. 5 are from samples in which the concentrations of the same three elements — cerium, praseodymium, and neodymium — were varied. The total weight of elements in each tag was held constant; however,

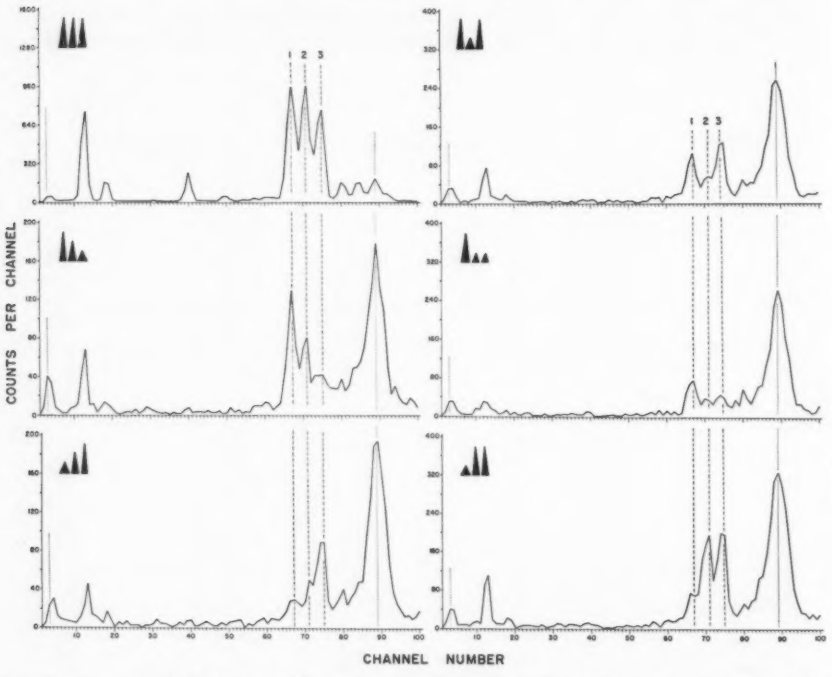

FIG. 5. Spectrographs of tags with varying concentrations of the elements cerium, praseodymium, and neodymium. The figure at the upper left is a spectrograph of the elastomer mixture of the three elements in the absence of fish tissue (standard). The diagram in the upper left of each figure indicates the approximate relative concentrations. All samples were exposed for 8 min. Identification of X-ray peaks: 1 — CeK_α, 2 — PrK_α, 3 — NdK_α.

the proportions were varied to give four classes. The number of tags theoretically possible in this case is 4^3 or 64. In practice fewer combinations are possible, for although one can detect relative concentrations, it is difficult to determine the exact concentration of the elements in the tissue. In addition, there is generally more work required to mix the tags to give peaks of different relative heights because equal weights of elements in a tag do not necessarily give peaks with the same height. In the graphs in Fig. 5, the abscissa has been expanded to include counts from all 100 channels.

BIOLOGICAL SIDE EFFECTS

Some possibly harmful biological side effects are associated with tissue damage due to the hypodermic needle, the toxicity of the silicone rubber and of the chemicals within the tag, and the effect of radiation on both the organism and personnel in the laboratory. Groups of fish tagged with different elements, and control groups injected with silicone rubber and with distilled water, have been reared for 6 months. Individuals from several of these samples were used to demonstrate the method (Fig. 2–5). Conclusions regarding the effects of the tag in the organism are still premature. However, histological sections of fish tissue surrounding tags show no evidence of tag rejection after 6 months and there is evidence that a connective tissue capsule has formed around the tag. Nevertheless, certain elements are likely to be toxic to the organism and if the silicone rubber does not isolate the chemicals effectively, it will be necessary to encapsulate the elements in some inert substance. This should present no real problem, but chemical encapsulation of the toxic elements will reduce the number of combinations possible in tags of restricted size.

Because ionizing radiations are used to decode the tag there are health hazards to both the specimens analyzed and to personnel using the apparatus. The effects of radiation damage depend upon the areas of the body exposed to the radiation, the total dose encountered, the particle type and energy of the ionizing radiations, and the dose rate. A given exposure produces less damage when distributed over a long period of time than when concentrated over a short period. In addition the effects of radiation are less serious when extremities rather than vital organs or the whole body is exposed with a given dose. These are problems that have been dealt with elsewhere and for details the following may be consulted: Quimby (1950), Griffin (1952), Lebedinsky et al. (1958), and Seymour (1958).

The ^{241}Am used in this study is a personnel hazard because of its long half-life (458 years) and because of its affinity for bone. This is especially true if it is ingested since within the bone small quantities can produce significant damage. Much of this hazard has been reduced by obtaining the ^{241}Am already sealed within the source assembly. Accidental rupture of the seal is possible, however, and the potential hazards from ingestion could be realized if monitoring and safety precautions were neglected. Fortunately, the amount of radioactivity needed to decode the tags (20 mCi) is relatively small.

There are two sets of standards used for determining the maximum radiation dose permitted for people: those that apply to workers using radioactivity, and those that apply to the population as a whole. Personnel operating the equipment in this laboratory have never accumulated more than 2 mR of whole body exposure per month, even though the unshielded equipment has been in virtually continuous operation. This value is approximately 0.5% of the maximum permissible dosage for that period for workers as set by the Atomic Energy Control Board of Canada (see Department of National Health and Welfare, 1966; Morgan, 1962).

Fish were tagged near the adipose fin, above the lateral line (Fig. 6). The amount of radiation received by fish in the process of decoding the tags was measured to be 3 mR/min. Thus, if the time required to decode the tag is a minute or less, the total exposure to the fish is less than 3 mR. In addition, this amount of time out of water should not be harmful to the fish. For comparison, the dosage received during medical X-ray diagnostics is from 60 mR to 6000 mR per exposure. Furthermore, for medical application the X-ray energy is higher than those used for the tag and the dose is given during a much shorter time interval (Recommendations of the International Commission on Radiological Protection, 1955).

DISCUSSION AND CONCLUSIONS

Several techniques for rapid qualitative and quantitative chemical analysis are available that allow for detection of elements in concentrations of 10 ppm or less. These techniques provide marking methods that employ the presence or absence, or relative concentrations, or both, of chemical elements as a form of code.

Many different techniques have been used successfully in the past to incorporate foreign elements in the tissue of the organism to be tagged: feeding, implanting, and immersion in solutions (Ophel and Judd, 1968; Phinney et al., 1967; Scott, 1962). We have successfully introduced easily detectable amounts of cadmium, strontium, and barium by mixing lactate compounds of these elements in the diet of specimens. We have also detected with X-ray fluorescence the magnetic wire tags of Jefferts et al. (1963) in vivo; these were coated with lanthanum oxide. In general, many of the earlier methods of chemical marking may be rendered more useful if the X-ray fluorescence method or related detection methods are employed.

The results of the present study were obtained by using a tagging scheme in which elements were mixed in silicone rubber and injected in liquid form into the flesh of sockeye salmon. In this manner it was possible to generate a large number of tags that were easily distinguished in the tissue. If one employs a binary system in which chemical elements are either included or excluded as a form of code, the number of distinct tags using n elements is 2^n. The number of possible tags with n elements increases if the relative concentrations of the elements present are also varied. In this instance the number of combinations is k^n where k is the number of distinct classes or concentrations. One might speculate that at least 2^{25} different tags are possible and perhaps as many as 4^{25}.

Unfortunately, every addition of an element reduces the concentration of each element in the tag if the maximum size of the tag is fixed. One may circumvent the problem of critical concentration by using larger tags, but this in turn will place restrictions on the minimum size of the organism to be tagged. The concentration of lanthanum was reduced to $\frac{1}{10}$ of that used in the study to approximate amounts that would be present in mixtures. The element was still easily detectable at this concentration. Mixtures of 10

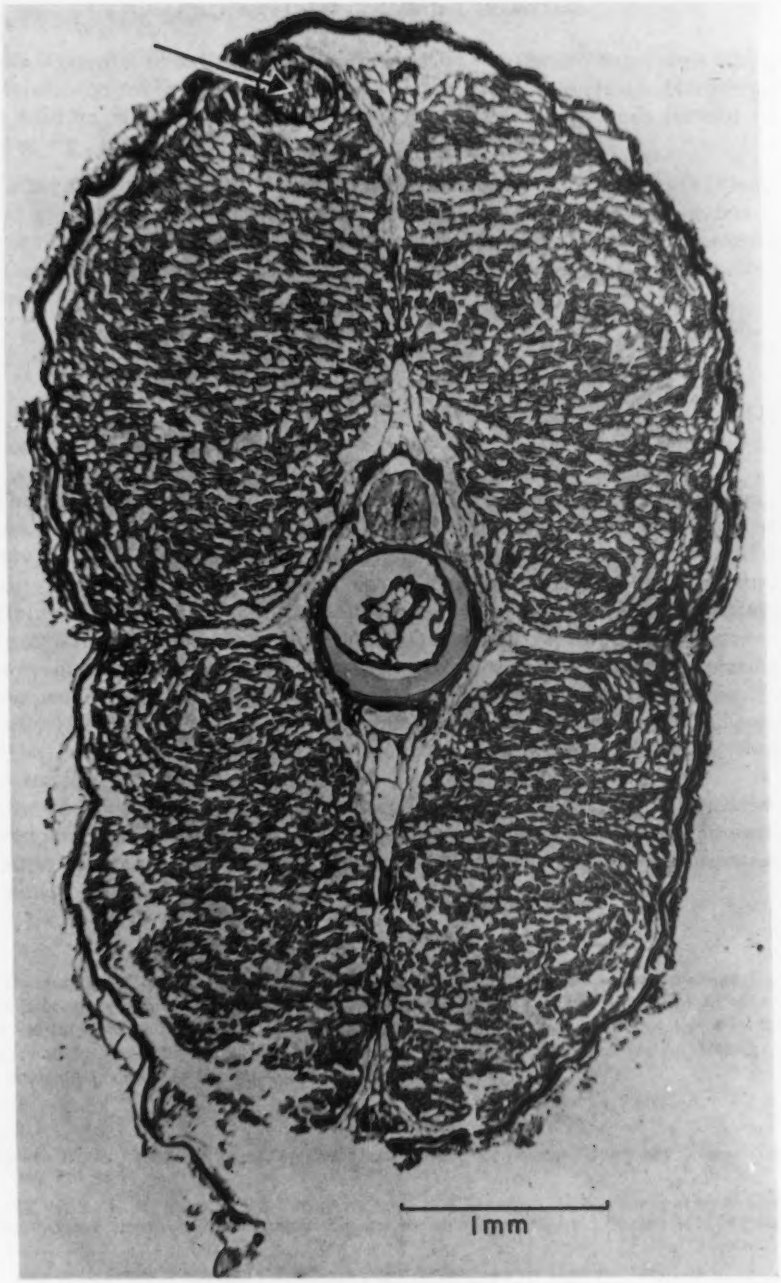

FIG. 6. Cross section of a sockeye salmon fingerling through the adipose fin. The arrow points to the silicone rubber tag. The tagged area has been outlined in ink.

elements were attempted, but were hampered by reaction amongst each other, and the silicone rubber hardened before the tag could be injected. Hardening of the silicone might be avoided by choosing stable compounds instead of elements, e.g., silver iodide.

To keep the tag small it is possible to include only a few elements in any tag and still construct a large number of combinations providing one has a large number of different elements to choose from. If one chooses r elements for a tag from a group of n possible elements, the number of distinct combinations is

$$C = \frac{n!}{(n-r)!r!}$$

where $n! = n \cdot (n-1) \ldots 1$. Here we see that a maximum of 298 tags is possible using only 12 different elements and without using more than 3 elements in any one tag.[2]

The fish used in this study were individuals preserved soon after marking. Samples from the same experimental groups have since been examined at monthly intervals and show no obvious signs of adverse effects, i.e., differential mortality, nor have we experienced any difficulty in reading the tags and identifying the fish. Although tags appear to be encapsulated in the tissue, it is premature to draw any conclusions regarding rejection or retention. In the event that the silicone rubber carrier proves to be ineffective in isolating the compounds, encapsulation with another inert material may be necessary. Presently 150 different groups of pink salmon are being marked as they emerge from the gravel where they hatched. In these studies we are finding that fish as small as 2.5 cm are easily tagged.

One additional feature of the tag decoding scheme is that the information is stored and transmitted electronically in a format compatible with computers. Computer programs are now being developed that will reduce the information to a binary number.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of staff members at the Lawrence Radiation Laboratory, University of California, Berkeley, Calif. Mr Lichliter arranged loan of the source and target assembly and Mr R. Giauque provided valuable suggestions during the initial feasibility study. Drs John Mason and Robert Parker of the FRB Biological Station, Nanaimo, B.C., read initial drafts of the manuscript.

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[2]The number 298 is obtained by successive substitution of 3, 2, and 1 for "r" in the equation and then summing the three results.

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Effects of Sublethal DDT on a Simple Reflex in Brook Trout

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Anderson, J. M., and H. B. Prins. 1970. Effects of sublethal DDT on a simple reflex in brook trout. J. Fish. Res. Bd. Canada 27: 331–334.

Brook trout were conditioned in about 30 trials to exhibit the propeller-tail reflex, with electric shock as the unconditioned stimulus and light as the conditioning stimulus. When the trout were exposed for 24 hr to sublethal DDT (20 ppb), more than half could not be conditioned at all; the remainder required significantly more trials than did the untreated ones. Three to four weeks after initial testing, the DDT effect had largely disappeared. It seemed likely that the DDT was acting upon central nervous system structures. The general ecological implications of the results are discussed.

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INTRODUCTION

Fish that have been exposed to very low doses of DDT may seem perfectly normal to the casual observer. Experiments, however, often reveal profound physiological and behavioural disturbances. Changes have been observed in temperature selection (Ogilvie and Anderson, 1965; Javaid, MS, 1967), lower lethal temperature (Elson and Kerswill, 1966; Elson, 1967), lateral line function (Anderson, 1968), EEG pattern of the cerebellum (Aubin and Johansen, 1969), and the cold-blocking temperature of a simple reflex (Anderson and Peterson, 1969).

Recent work suggests that instrumental learning may also be affected by sublethal DDT (Anderson and Peterson, 1969). However, the failure to establish a simple visual-conditioned avoidance response in these experiments, using a standard shuttle box apparatus and techniques, could be accounted for by either sensory or motor impairment, or both; that is, the results did not seem to warrant the generalization that learning in the usually accepted sense, i.e. changes in the central nervous system, was being affected by the DDT.

The experiments reported here deal with the effect of sublethal DDT on a conditioned response involving an extremely simple reflex (the propeller-tail reflex). The greatly reduced complexity of the sensory and motor apparatus required to exhibit this response would make any observed changes in the rate of learning much more likely to be a function of the central nervous system.

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MATERIALS AND METHODS

Mild stimulation of the gular region of fish results in a weak propellerlike movement of the tail (von Holst, 1934). This simple reflex was elicited using mild electric shock as unconditioned stimulus (US). Light was the conditioned stimulus (CS) and the experiment was designed to test whether previous exposure to DDT affected the rate of establishment of the conditioned response. The apparatus and experimental protocol are depicted in Fig. 1.

The CS was delivered from a Grass P8 stimulator and consisted of a 500 msec train of square wave pulses (1 msec and approximately 2.5 v each at a rate of 400 per sec). The learning criterion was five consecutive correct responses, a "correct response" being the reflex appearing after the CS but before application of the US. If conditioning was not established by 100 trials, the experiment was discontinued.

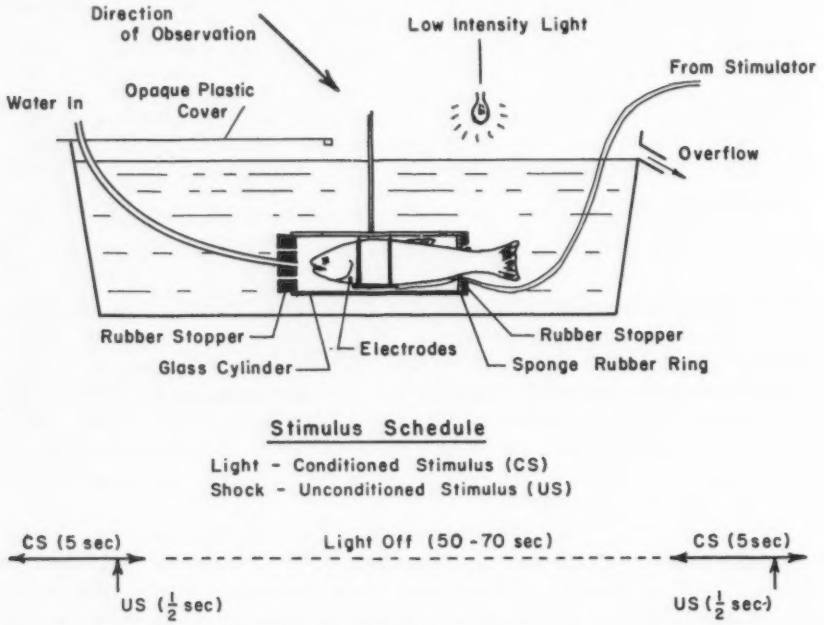

FIG. 1. Apparatus and experimental protocol used in conditioning experiments.

The fish were brook trout (*Salvelinus fontinalis* Mitchill) and were approximately 1.5 years old at time of testing. Weight ranged from 110 to 180 g. They were fed DDT-free beef liver once daily. To minimize the amount of detritus present, the fish were not fed for 3 days prior to and during the period of treatment. Fish were exposed to 20 ppb of DDT for 24 hr in 9 liters of continuously aerated water in a glass jar, one fish per jar. The temperatures for acclimation, exposure, and testing were the same, 12 ± 1.0 C. DDT was added to the water in 0.45 ml acetone. Control fish were treated the same as were the experimental ones, except that for one half only acetone was added and for the other half no acetone was added. All testing was done during daylight hours, in clean water immediately after the 24 hr exposure to DDT.

RESULTS

There was no difference between the results for the acetone and no-acetone controls. The data have therefore been combined. Of the 16 fish

comprising the control group, all fish, with two exceptions (at 51 and 90 trials), took no more than 50 trials to be conditioned (Fig. 2). The average was 29 trials. In contrast, 10 of the 16 DDT-treated fish failed to become conditioned after 100 trials and the remainder took 60 or more trials, with an average of 76 trials. Clearly DDT treatment prior to training markedly affected the rate of learning of the propeller-tail reflex.

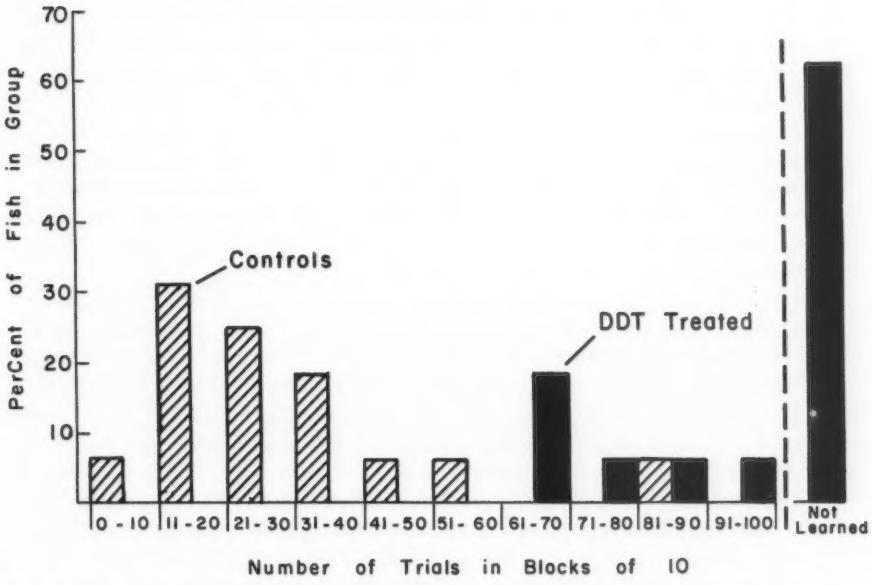

FIG. 2. Effects of 20 ppb DDT on the establishment of the conditioned response. The number of trials needed to reach training criterion have been grouped into blocks of 10.

It had been planned to retest all of the fish at various intervals after first training to investigate the permanency of the DDT effect. However, the holding apparatus put the fish under stress. Scale loss of the fish after removal from the apparatus was particularly heavy. Unfortunately, therefore, the survival of fish after the initial experiments was not good.

The results from fish that did survive, however, are revealing. All retesting was done from 3 to 4 weeks after the trials. Four control fish survived. At first testing their average number of trials was 28; at second testing it was 27. (The latter value would have been lower, as expected, were it not for one fish that took 11 more trials than initially. The remaining fish took 8 or 9 trials less.) Of the 9 surviving DDT-treated fish, 4 at first testing had not been trained by 100 trials; the remainder had an average of 73 trials. At second testing, although one of the original 4 was still not trained by 100 trials, all the rest were trained in significantly fewer trials; the average was 38.

DISCUSSION

Classical conditioning (of the propeller-tail reflex) is made markedly more difficult by prior exposure of the fish to sublethal DDT. Unlike the previous work dealing with instrumental learning, the chances of motor impairment being responsible for the results seem remote. However, it was observed that the US could elicit the propeller-tail reflex at a lower voltage for the DDT-treated fish than for the control ones. It seems unlikely, however, that a heightened sensitivity of receptors (if real) could account for the drastic decrease in learning rate. A more likely possibility to us is that the DDT is directly affecting the normal functioning of the central nervous system, perhaps in the region of the optic tectum.

This work, and that reported elsewhere on the sublethal effects of DDT, suggest that insecticide pollution may be even more harmful to fish than the well-publicized lethal effects that have been reported. Learning, for example, is probably a critical element in such behavioural activities as territorial defence and migration. Any pollutant that might affect such behaviour patterns is obviously of potential ecological significance.

To the extent that insecticide-induced changes in behaviour may adversely influence the survival of the species, the term "sublethal," applying to the concentrations used, is clearly a misnomer, or at any rate may imply to some that no biological effects of consequence are involved. Perhaps "subacute" would be a more appropriate term. As reemphasized recently by Macek (1968), no-effect levels, rather than no-kill levels, should serve as the basis for establishing water quality criteria.

The fact that the effects of the 24-hr exposure to DDT in these experiments had largely disappeared within 3–4 weeks is of course encouraging. The only disquieting aspect is the knowledge that in nature there are many areas where fish are unable to avoid continuous exposure to low doses of insecticides.

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Enhancement by Glycerol of Phototrophic Growth of Marine Planktonic Algae and its Significance to the Ecology of Glycerol Pollution

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The effects of low (0.05 M) and high (0.5–1.0 M) concentrations of glycerol on the growth of 18 species of marine phytoplankters belonging to 7 algal classes were studied. The algae were grown in axenic culture in sea water enriched with nitrate, orthophosphate, silicate, trace-metal ions, vitamin B_{12}, thiamine, biotin, and buffered at *p*H 7.6–7.8. Apart from a chrysomonad (*Prymnesium parvum*) and a cryptomonad (*Chroomonas salina*), none of the species showed any significant growth on glycerol in the absence of light. However, in the presence of light, glycerol enhanced the growth of 16 species, in particular members of the Chrysophyceae and Cryptophyceae, one diatom (*Phaeodactylum tricornutum*), one rhodophyte (*Porphyridium cruentum*), and one chlorophyte (*Nannochloris oculata*). A high concentration of glycerol was required for inducing or asserting growth enhancement of certain species, but was equally effective as the low concentration or was inhibitory to other species. The enhancement effect was observed in the growth rate and in the peak population density, which in many instances was several times that from nonglycerinated cultures and suggested photoheterotrophic growth. Some species showed obvious cytological and metabolic changes from growth on glycerol. The ecological implications of glycerol pollution of sea water are discussed.

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INTRODUCTION

OUR INTEREST in the area of organic pollutants in sea water has prompted us to examine the effects of glycerol on the growth of marine phytoplankters. This study was particularly desirable in view of a recent report (Rahat and Jahn, 1965) of chemoheterotrophic[1] growth of a marine chrysomonad (*Prymnesium parvum*, previously considered to be a strict photoautotroph), requiring unusually high concentrations (0.2–0.5 M) of glycerol; the growth was specific to glycerol, and low concentrations, unable to support growth in darkness, strongly enhanced growth in the light (Rahat and Spira, 1967). An obvious implication of this observation is that glycerol pollution may stimulate blooms of glycerol-responsive algae even in the absence of light, with consequent

[1]The following terms are used to denote modes of heterotrophic growth: *chemoheterotrophy*, growth in darkness on organic compounds serving as carbon and energy sources; *photoheterotrophy*, growth in presence of light on organic compounds, with light providing the bulk of the energy and the organic compound a considerable proportion of the carbon.

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effects on established ecological systems. Apart from this consideration, the possibility of metabolism of extraneous glycerol by unicellular algae is expected to be of considerable biochemical value in understanding control mechanisms of lipogenesis and gluconeogenesis within the algal cell, since earlier investigators have concentrated on algal heterotrophy of standard sugars, acetate, lactate, glycollate, Krebs-cycle intermediates, amino and fatty acids, and have virtually ignored glycerol (Danforth, 1962; Pintner and Provasoli, 1963; Droop and McGill, 1966; Bunt, 1969).

In the present investigation, we have studied the effects of a low (0.05 M) and two high (0.5, 1.0 M) concentrations of glycerol on the growth of 18 unicellular species of marine phytoplankters belonging to 7 algal classes. The algae were grown axenically under constant conditions of continuous illumination or darkness at 20 C in a buffered seawater medium enriched with inorganic nutrients and vitamins, and growth was followed by periodic cell counts, turbidimetric measurements, and microscopic observations.

MATERIALS AND METHODS

Algal Species

All the species studied were obtained as axenic or unialgal specimens from various culture collections and have been maintained in axenic culture in this laboratory for a considerable period of time; information on their source (original isolate, name of specimen-culture donor) is appended below in parentheses after each species.

CHLOROPHYCEAE

Dunaliella tertiolecta Butcher (Woods Hole clone "Dun," Dr J. D. H. Strickland); *Tetraselmis maculata* Butcher (Nanaimo strain "TMD," Dr T. R. Parsons); *Nannochloris oculata* Droop (Millport strain no. 66, Dr M. R. Droop); *Brachiomonas submarina* (Bohlin) Droop var. *pulsifera* 7/2a (Millport strain no. 44, Dr M. R. Droop).

CHRYSOPHYCEAE

Monochrysis lutheri Droop (Millport strain no. 60, Dr J. D. H. Strickland); *Isochrysis galbana* Parke (Woods Hole clone "Iso," Dr J. D. H. Strickland); *Coccolithus huxleyi* (Lohm) Kamptner (Woods Hole clone "BT-6," Dr T. R. Parsons); *Prymnesium parvum* Carter (Reich's Israeli strain, Dr M. Rahat).

BACILLARIOPHYCEAE

Phaeodactylum tricornutum Bohlin (Lewin strain 74-M, Dr J. C. Lewin); *Skeletonema costatum* (Grev.) Cleve (Woods Hole clone "Skel," Dr R. R. L. Guillard); *Cyclotella nana* Hustedt (Woods Hole clone "3H," Dr R. R. L. Guillard).

CRYPTOPHYCEAE

Chroomonas salina (Wislouch) Butcher (Woods Hole undetermined cryptomonad clone "3C," Dr F. T. Haxo); *Rhodomonas lens* Pascher and Ruttner (Lasker's Gulf-stream strain, Dr L Provasoli); *Hemiselmis virescens* Droop (Millport strain no. 64, Dr L. Provasoli).

DINOPHYCEAE

Amphidinium carteri Hulburt (Woods Hole clone "Amphi 1," Dr L. Provasoli).

RHODOPHYCEAE

Porphyridium cruentum Nägeli (Vischer's strain no. 107, Dr F. T. Haxo).

CYANOPHYCEAE

Agmenellum quadruplicatum (Menegh.) Brébisson (Van Baalen's strain "PR-6," Dr C. Van Baalen); *Anacystis marina* (Hansg.) Drouet and Daily (Van Baalen's strain "6," Dr C. Van Baalen).

Growth Tests with Glycerol

All tests were conducted in a basal medium obtained by enriching the stock-culture maintenance medium with additional nitrate (2 mM) and phosphate (0.2 mM) after autoclaving (Table 1). The nitrate was replaced by glycine (4 mM) in the case of *Hemiselmis virescens* because of its inability to utilize nitrate as N-source for growth (Antia and Chorney, 1968). Appropriate aliquots of an autoclaved 10-M aqueous glycerol stock solution were added to the autoclaved basal medium.

Table 1. Composition of stock-culture maintenance medium and basal medium used for growth studies with glycerol.

	Maintenance	Basal
KNO_3	50 mg (500 μmoles)	250 mg[a] (2.5 mmoles)
$NaH_2PO_4 \cdot H_2O$	6.9 mg (50 μmoles)	34.5 mg[a] (250 μmoles)
$Na_2SiO_3 \cdot 9H_2O$	84 mg[a] (300 μmoles)	
Vitamins:		
Thiamine·HCl	0.5 mg (14.8×10^{-1} μmoles)	
Biotin	1 μg (4.1×10^{-3} μmoles)	
B_{12}	2 μg (13.4×10^{-4} μmoles)	
Trace metals (chelated)[a]:		
$Na_2 \cdot EDTA \cdot 2H_2O$	8.1 mg (21.8 μmoles)	
$FeCl_3 \cdot 6H_2O$	2.7 mg (10.0 μmoles)	
$MnSO_4 \cdot 4H_2O$	1.125 mg (5.0 μmoles)	
$ZnSO_4 \cdot 7H_2O$	0.575 mg (2.0 μmoles)	
$Na_2MoO_4 \cdot 2H_2O$	0.243 mg (1.0 μmole)	
$CuSO_4 \cdot 5H_2O$	0.025 mg (0.1 μmole)	
$CoSO_4 \cdot 7H_2O$	0.014 mg (0.05 μmole)	
Buffer:		
Tris·HCl { 41.3 mM, *p*H 6.8–6.9 *before* autoclaving[b] }	200 ml (1 g or 8.3 mmoles Tris)	
Sea water, open ocean, salinity 33‰	To 1 liter	

[a] *p*H of stock solutions of these components was adjusted to 7.6–7.8 with aqueous HCl or NaOH before incorporation with the rest of the medium.
[b] This gives a *p*H of 7.6–7.8 in the medium *after* autoclaving (15 min at 120 C).

The growth tests were carried out in 2 ways: (A) 40-ml aliquots of basal medium containing 0, 0.05, and 0.5 M glycerol in 125-ml capacity screw-capped Erlenmeyer flasks were generally inoculated with ca. 1×10^6 algal cells from a 7–10-day-old photoautotrophically grown stock culture, and the flasks were incubated stationary in continuous light (from cool-white fluorescent lamps providing illumination of ca. 250–300 ft-c intensity) or darkness at 20 ± 2 C, with mechanical agitation for a few minutes once every 24 hr. Growth measurements were made weekly on 1-ml culture samples by determining the optical density at 600 mμ (Beckman DU spectrophotometer) and by counting the algal cell concentration on an AO Spencer bright-line hemacytometer. (B) 4-ml aliquots of basal medium containing 0, 0.05, 0.5, and 1.0 M glycerol in 8-ml capacity screw-capped culture tubes (chosen to be optically clear) were inoculated with 10^5–10^6 algal cells and incubated in light as above. Growth measurements were made daily on intact cultures by determining the optical density at 600 mμ (Bausch and Lomb Spectronic 20), and relative growth-rate constants (Table 2) were calculated as the increase in optical density per 24 hr from the steepest rectilinear slopes of growth rate curves (Fig. 1).

TABLE 2. Summary of effects of glycerol on phototrophic growth of 18 species of marine planktonic algae.

Agal species	Glycerol concn (M)	Cell concn ($\times 10^6$ cells/ml) at following growth periods (*days*) 7	14	21	28	Relative growth rate constant ($\times 10^{-2}$) (see text)	Reaction to iodine after 28 days' growth[a]	Cytological observations: Cell size range (spherical or ellipsoid dimensions, μ)	Cytological observations: Obvious abnormalities due to glycerol
CHLOROPHYCEAE									
B. submarina	0	0.2	0.3	0.4	0.4	2	−	8–20×6–16	Nil
	0.05	0.2	0.2	0.3	0.4	2	−		
	0.50	0.2	0.2	0.3	0.3	1	−		
D. tertiolecta	0	1.0	1.4	1.9	2.1	80	−	14–16×8–10	Nil
	0.05	1.2	1.7	2.1	2.7	75	−		
	0.50	1.1	1.9	2.0	2.8	88	−		
N. oculata	0	6.6	23.0	37.5	49.0	17	−	2–3	Nil
	0.05	4.9	22.3	38.6	58.8	33	−		
	0.50	3.4	25.9	61.4	126.3	72	−		
T. maculata	0	0.4	1.0	1.1	1.4	74	+	12–16×10–12	Nil
	0.05	0.9	2.7	5.3	6.7	89	++		
	0.50	0.4	0.8	1.2	2.1	34	+		
CHRYSOPHYCEAE									
I. galbana	0	2.5	4.9	6.7	7.9	19	−	4–6 ×3–5	Nil
	0.05	4.0	16.6	33.6	34.8	101	−		
	0.50	2.4	14.5	36.4	37.2	120	−		
P. parvum	0	1.4	2.4	3.1	3.8	54	−	10–12×6–8	Gigantism, with massive enlargement of leucosin (?) vesicles (Fig. 2, 3).
	0.05	2.2	4.9	7.3	11.1	115	−	10–14×6–10	
	0.50	5.0	13.4	1.2	0	246	−	14–16×10–12	
M. lutheri	0	1.6	2.8	4.4	5.9	39	−	4–6×3–4	Nil
	0.05	1.7	4.9	7.9	15.4	85	−		
	0.50	1.7	4.8	7.6	14.2	66	−		
C. huxleyi	0	4.8	7.7	8.8	9.2	9	−	2–3	Marked increase in intra- and extra-cellular refractile particles.
	0.05	5.0	10.6	11.5	15.1	17	−	2–3	
	0.50	<0.1	6.2	26.6	4.3	51	−	3–5	
BACILLARIOPHYCEAE									
P. tricornutum	0	1.7	4.4	5.8	6.6	14	−	21×4 (spindle); 8–4 (oval)[b]	Nil
	0.05	4.0	19.3	48.0	52.8	95	−		
	0.50	2.4	10.8	35.4	46.2	85	−		
C. nana	0	3.2	5.3	6.6	5.0	10	−	4–6×6–8	Nil
	0.05	3.8	6.6	7.4	5.9	32	−		
	0.50	3.8	6.8	7.4	6.6	32	−	4–5×6–12	Promotion of cell elongation
S. costatum	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5–6×10–18	

	0.05	4.0	19.3	48.0	52.8	95	–	21×4 (spindle);	Nil
	0.50	2.4	10.8	35.4	46.2	85	–	8–4 (oval)[b]	
C. nana	0	3.2	5.3	6.6	5.0	10	–		
	0.05	3.8	6.6	7.4	5.9	32	–	4–6×6–8	Nil
S. costatum	0.50	3.8	5.3	7.4	6.2	30	–	4–5×8–12	Promotion of cell
	0.05	4.5	2.6	0	0	85	–	5–6×10–18	elongation.
	0.50	<0.1	<0.1	0.9	0	0	–	5–6×10–18	
CRYPTOPHYCEAE									
C. salina	0	0.8	1.3	1.5	1.4	56	–	8–12×4–8	Gigantism, with intra- and extracellular accumulation of iodine-stainable particles (Fig. 4–6).
	0.05	1.9	4.0	2.8	1.5	128	++	14–20×8–10	
	0.50	1.7	1.1	1.4	1.2	171	++	14–20×8–10	
R. lens	0	0.8	1.8	2.4	2.9	48	–	12–14×6–8	Cell enlargement with intracellular accumulation of refractile iodine-stainable particles.
	0.05	3.4	4.3	2.2	0.9	139	++	14–16×8–10	
	0.50	<0.1	1.0	3.4	0.8	133	++	18–20×10–12	
H. virescens	0	3.9	5.9	7.0	3.6	68	–	4–5×6–8	Gigantism, with extracellular release of crescent-shaped iodine-stainable granules (Fig. 7–9).
	0.05	7.1	11.6	12.7	9.5	162	++	4–5×6–8	
	0.50	1.7	7.4	4.3	<0.1	153	++	8–10×10–16	
DINOPHYCEAE									
A. carteri	0	0.3	0.4	0.6	0.7	37	–	10–12×14–16	Swollen cells with intracellular accumulation of "assimilate" granules[d] (Fig. 10, 11).
	0.05	0.4	1.0	1.6	1.9	114	+[c]	10–14×16–18	
	0.50	<0.1	<0.1	<0.1	<0.1	0	–	Nil	
RHODOPHYCEAE									
P. cruentum	0	0.3	0.5	0.7	0.9	22	–	4–8	Nil
	0.05	0.2	0.9	1.1	1.6	22	tr	4–10	
	0.50	0.3	8.1	8.8	7.8	115	++	4–10	
CYANOPHYCEAE									
A. quadruplicatum	0	15.8	28.3	59.8	86.0	17	–		
	0.05	7.2	27.2	78.3	133.0	38	–	2×2–4	Nil
	0.50	<0.3	1.3	28.6	64.0	15	–		
A. marina	0	0.08[e]	0.14[e]	0.22[e]	0.30[e]	11	–		
	0.05	0[e]	0.11[e]	0.28[e]	0.50[e]	32	–	0.8–1.0	Nil
	0.50	0[e]	0[e]	0[e]	0.01[e]	0	–		

[a]Key to abbreviations used: –, negative; tr, doubtful trace; +, weakly positive; ++, moderately to strongly positive.
[b]Spindle and oval forms of *P. tricornutum* as described by Lewin et al. (1958).
[c]Reddish-purple color developed unusually slowly and remained localized at the interface between aqueous and ether layers (see text).
[d]Described by Hulburt (1957).
[e]Optical density values at 600 mμ.

Periodic sterility checks for microbial contamination were made throughout the studies, using the STP medium of Provasoli et al. (1957).

IODINE REAGENT TEST FOR STARCH-TYPE POLYSACCHARIDE

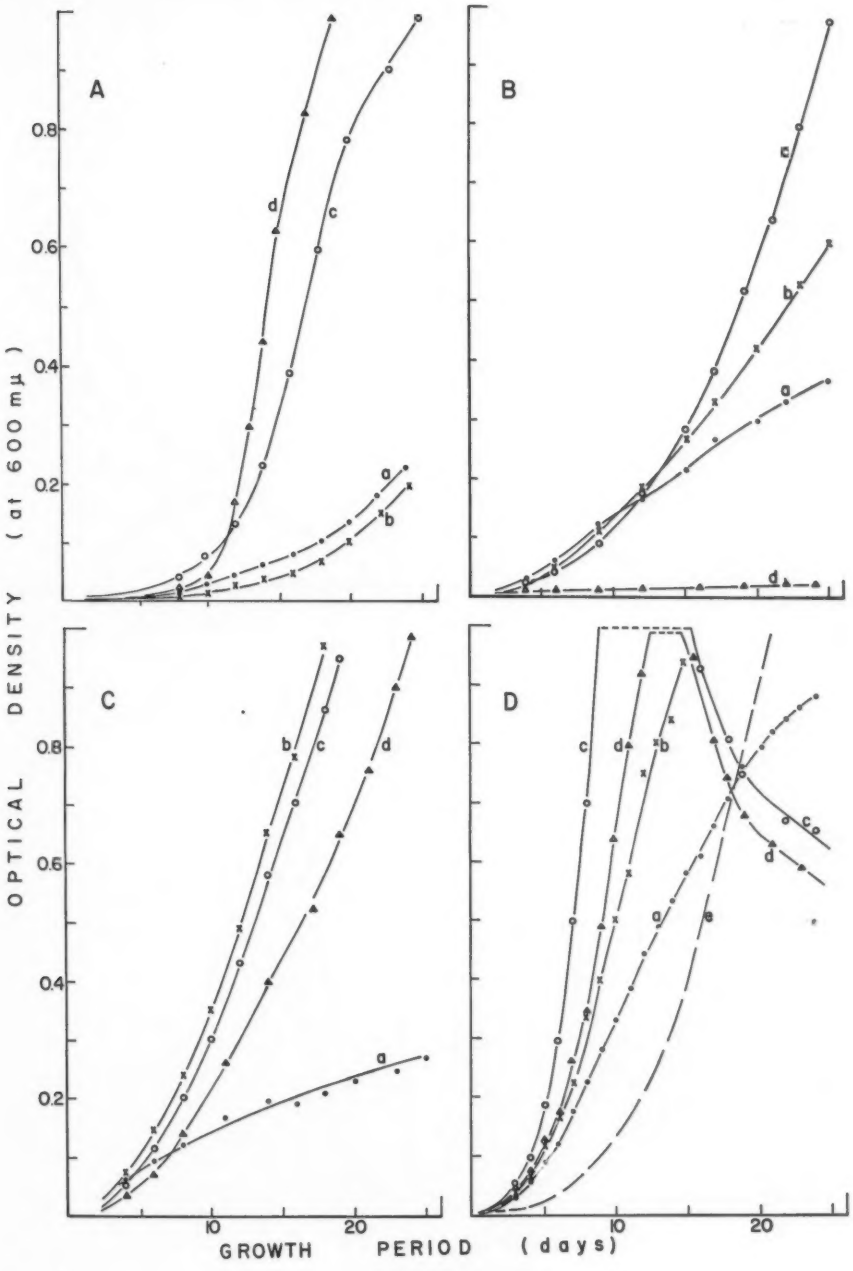
Aliquots (3–4 ml) of terminal cultures were treated with 2 drops of an aqueous solution of 0.1 M iodine and 0.24 M potassium iodide at 80–100 C for 15–20 min and cooled to room temperature. After adding another drop of the iodine reagent, the chloroplast pigments were extracted with an equal volume of ether, and the appearance of a blue to purple color in the aqueous phase was regarded as a positive reaction from starch-type polysaccharide (Table 2).

RESULTS AND DISCUSSION

The results of these studies are depicted in Table 2 and Fig. 1. Under continuous illumination, the two chlorophytes *D. tertiolecta* and *B. submarina* showed no significant change in growth physiology and morphology whereas the other algae were considerably affected by one or both concentrations of glycerol. In general, the lower concentration (0.05 M) enhanced growth whereas the higher concentration (0.5 M) was stimulatory to some species and inhibitory to others. All the cryptomonads and chrysomonads reacted favorably to glycerol, but too few representatives were examined to permit any generalization on the response of algal classes.

Some interesting effects of glycerol concentration on phototrophic growth were observed. The rhodophyte *P. cruentum* was indifferent to 0.05 M, but showed 5-fold increase in growth rate and at least 8-fold increase in cell yield on 0.5 M glycerol, and these were further enhanced by 1 M glycerol (Fig. 1A). The chlorophyte *N. oculata* responded moderately to 0.05 M, but strikingly to 0.5 M glycerol; it could not, however, tolerate 1 M concentration (Fig. 1B). In both instances, the response appeared to gain strength after a prolonged period of adaptation. The diatom *P. tricornutum* responded early and strongly to all glycerol concentrations to give 7–8-fold increase in ultimate cell yield, and the response to 1 M was only slightly inferior to that from 0.5 M (Fig. 1C). All the chrysomonads showed favorable response to both concentrations (0.05, 0.5 M) of glycerol, with *P. parvum* and *I. galbana* displaying the most pronounced enhancement (5–6-fold increase) of growth rate and "peak" cell yield (Table 2, Fig. 1D, E). *Prymnesium parvum* preferred high glycerol concentrations (0.5, 1.0 M) and responded so swiftly that it appeared to be "spent out" with onset of rapid cell lysis after achieving early peak of growth. *Monochrysis lutheri* showed a similar, though less pronounced, pattern of growth response as *I. galbana* and both species appeared to accept stimulation from 1 M glycerol after a period of adaptation, whereas *C. huxleyi* was completely inhibited by this concentration though showing optimal growth with 0.5 M glycerol (Fig. 1F). All the cryptomonads showed equally strong favorable

FIG. 1. Rates of phototrophic growth of (A) *P. cruentum*, (B) *N. oculata*, (C) *P. tricornutum*, (D) *P. parvum*, (E) *I. galbana*, (F) *C. huxleyi*, (G) *C. salina*, (H) *H. virescens*, (J) *C. nana*, (K) *S. costatum*, (L) *A. carteri*, (M) *A. quadruplicatum*, on basal medium enriched with the following levels of glycerol concentration: (*a*) nil, (*b*) 0.05 M, (*c*) 0.5 M, (*d*) 1.0 M. Chemoheterotrophic growth on 0.5 M glycerol is shown by curve *e*. For details, see text.


A
B
C
D
a
b
c
d
e
0.2
0.4
0.6
0.8
10
20
OPTICAL DENSITY (at 600 mμ)
GROWTH PERIOD (days)

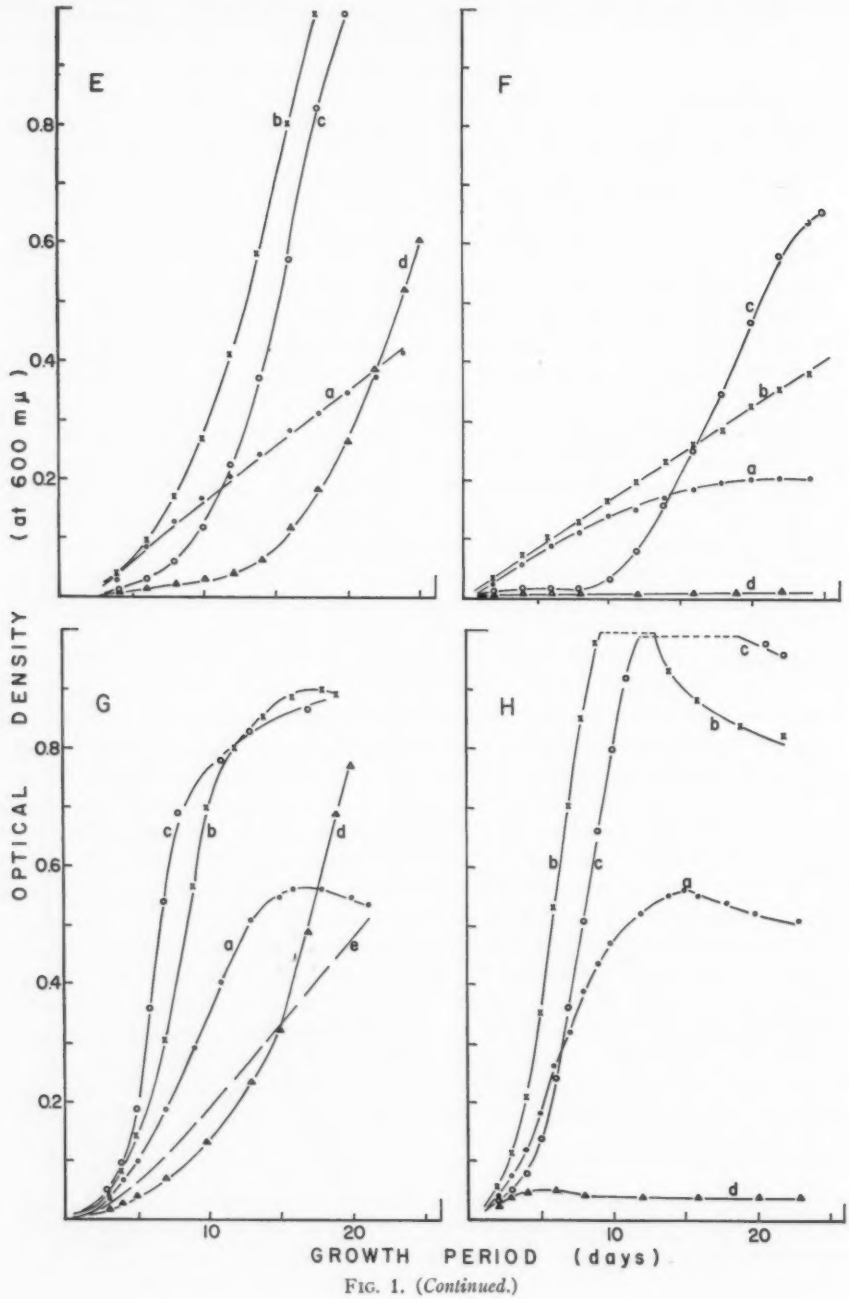


FIG. 1. (*Continued.*)

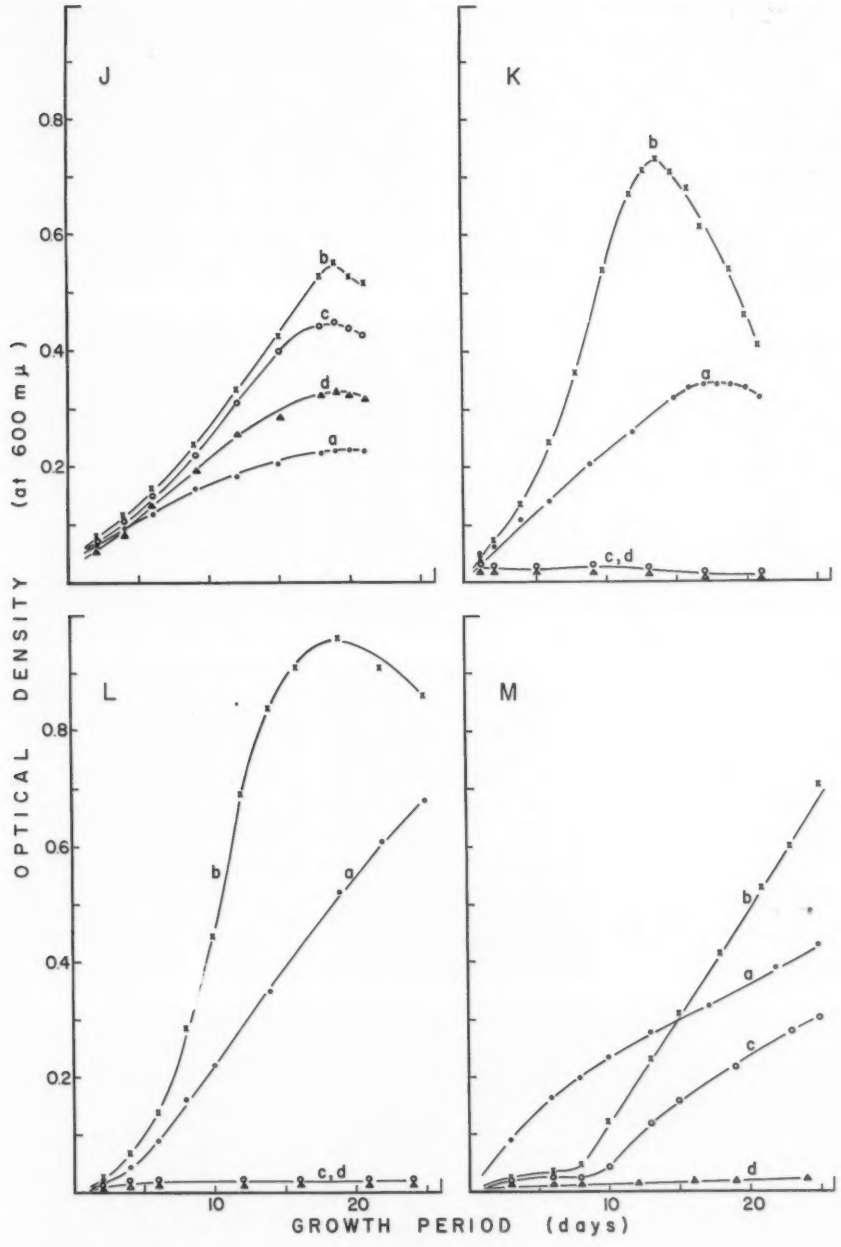


FIG. 1. *(Concluded.)*

response to both concentrations (0.05, 0.5 M) of glycerol; *C. salina* was exceptional in preferring the higher concentration and in adapting to beneficially tolerate 1 M glycerol, which appeared to be lethal to *R. lens* and *H. virescens* (Fig. 1G, H). The centric diatoms *C. nana* and *S. costatum* were markedly stimulated (3–4-fold) by the lower concentration, but displayed considerable difference in reaction to high glycerol concentrations (Fig. 1J, K). The dinoflagellate *A. carteri* was readily stimulated by 0.05 M, but could not cope with higher glycerol concentrations (Fig. 1L). Growth of the coccoid blue–green algae *A. quadruplicatum* and *A. marina* appeared to require adaptation before stimulation from 0.05 M glycerol, but was strongly suppressed by higher concentrations (Fig. 1M, Table 2).

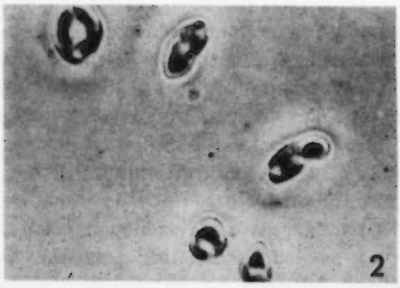
Apart from *P. parvum* and *C. salina*, none of the algae showed any indication of chemoheterotrophic growth on glycerol up to 4-weeks incubation in darkness; in doubtful instances, this incubation period was prolonged to 8 weeks. The absence of chemoheterotrophic growth was not due to loss of viability from prolonged exposure to darkness, since 90% of the dark-growth test cultures resumed growth on nontoxic glycerol concentrations after transfer to illumination. *Prymnesium parvum* and *C. salina* showed growth in darkness only on high glycerol concentration (their relative growth rates on 0.5 M glycerol are shown in Fig. 1D, G), thus confirming previously reported observations (Rahat and Jahn, 1965; Antia et al., 1969). We have maintained *C. salina* chemoheterotrophically by continuous 3-weekly subculture in darkness on 0.25 M glycerol for about 2 years and have observed that: (*a*) it retains pigmentation and copious starch-type polysaccharide production as reported earlier (Antia et al., 1969); and (*b*) it resumes normal growth in light on non-glycerinated maintenance medium without any obvious loss of its autotrophic nature.

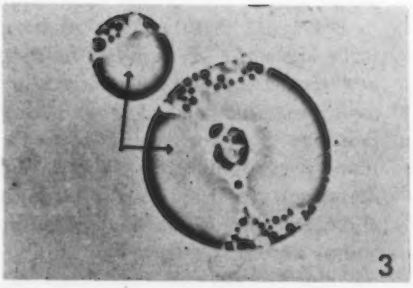
Several of the species responding to glycerol showed accompanying cytological abnormalities, whereas others, though strongly stimulated in growth, appeared to be unaffected, at least at the light-microscope level of observation (Table 2). In general, the cryptomonads showed a tendency to cell enlargement often resulting in "gigantism," with intracellular accumulation of "starch"

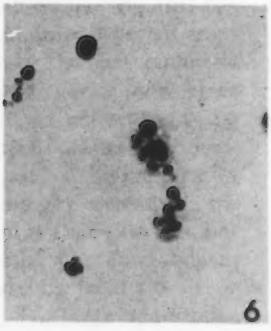
FIGURES 2–11.

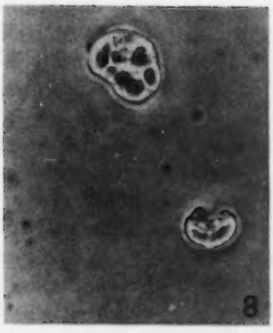
Glycerol-induced cytological changes of some phytoplankters observed under phase-contrast light microscope, ×1000. Cover slip pressure has caused some flattening of the large-sized cells.

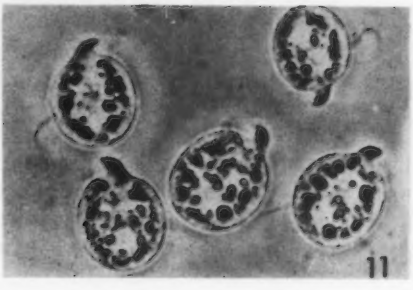
Fig. 2. Normal cells of *Prymnesium parvum* from photoautotrophic growth on basal medium. Fig. 3. Giant cells of *P. parvum* from growth on 0.5 M glycerol. The larger cell appears to be in process of division into four daughter cells. The arrows indicate enlarged leucosin (?) vesicles. Fig. 4. Normal cells of *Chroomonas salina* from photoautotrophic growth on basal medium. Fig. 5. Giant cells of *C. salina* from growth on 0.5 M glycerol; intracellular accumulation of opaque starch particles is evident. Fig. 6. Extracellular starch particles from glycerol-grown *C. salina*; stained with iodine reagent. Fig. 7. Normal cells of *Hemiselmis virescens* from photoautotrophic growth on basal medium. Fig. 8. Giant cells of *H. virescens* from growth on 0.5 M glycerol. Fig. 9. Extracellular crescent-shaped starch particles from glycerol-grown *H. virescens*; stained with iodine reagent. Fig. 10. Normal cells of *Amphidinium carteri* from photoautotrophic growth on basal medium. Fig. 11. Enlarged cells of *A. carteri* from growth on 0.05 M glycerol; intracellular accumulation of assimilate granules is evident.











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bodies and their eventual liberation from bursting of overgrown cells in aging cultures. This phenomenon has been reported by us (Antia et al., 1969) in some detail for *C. salina* and is further illustrated in Fig. 4–9. The cytological indication of starch "overproduction" by glycerol-grown cryptomonads was confirmed by strong positive reaction to iodine of aged bleached cultures unlike the nonglycerinated controls (Table 2). The iodine reaction also revealed increased starch production by *P. cruentum* and *T. maculata* from growth enhancement by glycerol, although their cytology appeared relatively unaltered. The dinoflagellate *A. carteri* showed a significant increase in cell size while accumulating an iodine-positive polysaccharide (Fig. 10, 11); the iodine reaction suggested an unusual lipid-bound starch or glycogen (Table 2). Among the chrysomonads, *P. parvum* was exceptional in showing "gigantism," with gross changes in cell structure (depicted in Fig. 2, 3) quite different from those displayed by the cryptomonads. As expected, none of the chrysomonads reacted positive to iodine, and it appears likely that the vesicular or particulate accumulations of *P. parvum* and *C. huxleyi* from growth on glycerol may be composed of "leucosin" or "paramylon" (laminarin-type polysaccharides).

The chemoheterotrophy displayed by *P. parvum* and *C. salina* leaves no doubt that these species can metabolize glycerol, and the excessive production of starch-type polysaccharide by *C. salina* indicates that this metabolism is strongly oriented towards gluconeogenesis. A similar photometabolism (or photoassimilation) of glycerol is indicated by the results from the other cryptomonads, despite their chemoheterotrophic inability. The large magnitudes of phototrophic growth enhancement of *P. cruentum*, *N. oculata*, *P. tricornutum*, *I. galbana*, and *A. carteri* also suggest considerable photoheterotrophic potential in these algae. The probability of glycerol promoting growth of some species from osmotic benefits (Guillard, 1963) without metabolism cannot be discounted, since the tests were carried out in a seawater medium of salinity (26‰, ionic strength equivalent to about 0.5 M NaCl) not necessarily ideal for optimal autotrophic growth of all species. Further studies with ^{14}C-labelled glycerol will be required to verify these inferences.

The stimulation of cryptomonad starch synthesis by glycerol recalls a recently reported instance (Thomas et al., 1969) of glycerol activation of higher-plant glucan synthesis involving a role of glycerol in allosteric regulation of glucan-synthesizing enzymes. A similar investigation of the cryptomonad glucan biosynthetic machinery would be highly desirable. In confirming the glycerol heterotrophy of *P. parvum* reported by Rahat and Jahn (1965) and Rahat and Spira (1967), we were surprised to note the cytological alterations previously not mentioned; the cytological manifestation of leucosin enhancement, if proved correct, may provide another instance of glycerol promotion of glucan synthesis. Our observation of the indifference of *D. tertiolecta* to glycerol was also surprising, since this chlorophyte was recently reported to produce considerable glycerol from photoautotrophic growth (Craigie and McLachlan, 1964); the supply of exogenous glycerol was expected to shift its photosynthetic metabolism toward storage-carbohydrate (starch has been

identified) production, but this was not observed. The strong growth enhancement of *P. tricornutum* noted by us is in sharp contrast to a previous observation (Hayward, 1968) of little effect from glycerol on phototrophic growth of this diatom; this discrepancy may be due to differences in the strains studied and in the culture conditions used.

Apart from their physiological and biochemical interest, the results of the present study predict an important ecological consequence of glycerol pollution of the marine environment. Depending on the availability of nitrate, phosphate, and vitamins, the introduction of glycerol into sea water is expected to "trigger off" blooms of a wide variety of phytoplankters and even to govern species succession in such outbreaks. The heterotrophic production of starch by cryptomonads may also have ecological significance and raises questions on the roles of algal heterotrophy and Cryptophyceae in oceanic starch production that has been previously attributed to autotrophic activities of Dinophyceae, Chlorophyceae, and benthic algae (Bursa, 1968).

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Influence of Body Weight on Chronic Oral DDT Toxicity in Coho Salmon[1]

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Median Survival Time was directly proportional to body weight in young coho salmon of the same age that were fed a diet containing technical DDT. Supplementation by additional feeding with DDT-free diet prolonged the lifespan of these fish but the Median Survival Time remained a direct function of body weight.

The primary effect of body size on lethality was to control the rate of DDT intake by the fish. The smallest fish consumed the greatest amounts of diet and, consequently, these fish received the highest milligram per kilogram doses of DDT.

In addition, however, the smaller salmon succumbed to a lower milligram per kilogram cumulative lethal dose than did the larger fish, perhaps because the smallest fish contained a lower percentage of lipid and thus failed to provide for adequate storage detoxification of the DDT.

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INTRODUCTION

RECENT TRIALS with juvenile chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon (Buhler et al., 1969) have demonstrated that tolerance to orally ingested DDT increases with increasing fish age or size. Not only was susceptibility to the pesticide greatest in the most immature fish but the smallest fish were affected first in a mixed population of the same age. The possibility of a size-selective mortality following exposure to pesticide could have considerable influence on natural fish populations containing a distribution of body sizes. Therefore, we have examined the influence of one variable, body size, in chronic lethality experiments in which young coho salmon of the same age, pooled in groups according to body weight, received technical DDT in the diet for periods as long as 125 days.

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METHODS

Fish used in this investigation were stock underyearling coho salmon (*O. kisutch*) of identical age from the Western Fish Nutrition Laboratory. Groups of experimental fish were maintained in individual troughs supplied with 9 C well water and fed in the manner described previously (Buhler et al., 1969). Fish were fed a synthetic diet[4] formulated to contain 200 ppm technical DDT[5] added in corn oil solution. A daily record of food consumption and mortalities was maintained for each group of fish.

Stock coho salmon were initially weighed individually in water by means of a Mettler precision top-loading balance and divided into 13 groups according to body weight (Table 1). Pooled groups of fish of equivalent size also were weighed at the beginning and upon termination of the experiment in order to establish the mean initial fish weight and the mean weight of the surviving fish.

TABLE 1. Distribution by weight of stock coho salmon.[a]

Group	Wt range (g)	No.	% of total
2	2.00–2.99	156	4.1
3	3.00–3.99	395	10.4
4	4.00–4.99	433	11.4
5	5.00–5.99	417	11.0
6	6.00–6.99	421	11.1
7	7.00–7.99	386	10.2
8	8.00–8.99	411	10.8
9	9.00–9.99	335	8.8
10	10.00–10.99	248	6.5
11	11.00–11.99	202	5.3
12	12.00–12.99	156	4.1
13	13.00 plus	238	6.3

[a]Total of 3798 fish weighed.

Groups of 78 or 100 fish of approximately equal size (groups 2A through 13A, Table 2) were fed to satiety three times daily for 123 days on a diet containing 200 ppm technical DDT. Additional lots of fish selected in the same manner (groups 2B through 13B, Table 3) received fixed amounts of the pesticide ration (based on the diet consumption of the smallest fish in preliminary trials and amounting initially to an intake of 0.04 mg of DDT per fish per day). After consumption of the daily allotted DDT diet, the B fish then were fed as much DDT-free ration as they would consume. The total amount of pesticide diet fed to each group of B fish daily was not reduced subsequently to compensate for mortalities as they occurred and, therefore, the daily DDT intake per fish then gradually increased during the experiment especially in those lots of fish exhibiting the greatest mortalities.

RESULTS

The plot of cumulative mortalities with respect to time for salmon fed DDT diet to satiation (A groups) is presented in Fig. 1. A standard log-prob-

[4]Experimental diets contained 35 g protein, 20 g dextrin, 2 g cod-liver oil, 8 g corn oil, 8 g vitamin mixture, 4 g mineral mixture, 23 g α-cellulose, and 10 ml water (Buhler et al., 1969).

[5]Entomological Society of American reference standard obtained from the City Chemical Corp., New York, and containing 77.2% *p,p'*-DDT [2,2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane].

TABLE 2. Effect on groups[a] of coho salmon fingerlings of uniform weight fed to satiation on a diet containing 200 ppm technical DDT.

Group	Avg initial wt (g)	MST[b] (*days*)	Approx daily DDT intake (*mg/kg per day*)[c]	Cumulative 50% lethal dose (*mg/kg*)[c,d]	Approx total DDT intake up to MST (*mg/fish*)
2A	2.89	31.0	3.07	95.0	0.33
3A	3.65	36.3	2.81	102	0.47
4A	4.59	46.3	2.29	105	0.64
5A	5.53	47.5	2.51	119	0.85
6A	6.51	55.0	2.33	128	1.10
7A	7.40	65.0	2.07	134	1.34
8A	8.44	72.2	1.94	140	1.61
9A	9.42	83.0	1.65	137	1.78
10A	10.46	89.3	1.46	130	1.86
11A	11.43	101	1.30	131	2.06
12A	12.49	104	1.38	143	2.46
13A	15.04	106	1.27	135	2.55

[a]Each group contained 100 fish, except groups 2A and 12A, which contained 78 fish.
[b]Median Survival Time.
[c]Based on a measurement of total diet consumption throughout the experiment and an estimation of mean fish weight at the MST. Estimation of the latter assumes a constant rate of fish growth between average initial to final fish weight.
[d]Approximate total DDT intake up to MST in mg/kg.

TABLE 3. Effects on groups[a] of coho salmon fingerlings of uniform weight fed a diet containing technical DDT and supplemented with control ration.

Group	Avg initial wt (g)	MST[b] (*days*)	Approx daily DDT intake (*mg/ kg per day*)[c]	Cumulative 50% lethal dose (*mg/kg*)[c,d]	Approx total DDT intake up to MST (*mg/fish*)
2B	2.94	29.5	3.48	103	0.38
3B	3.64	33.8	3.18	108	0.49
4B	4.55	42.3	2.90	122	0.72
5B	5.50	53.8	2.62	141	1.07
6B	6.48	63.8	2.39	153	1.42
7B	7.47	85.0	1.88	160	1.89
8B	8.40	97.5	1.61	157	2.06
9B	9.41	109	1.38	150	2.26
10B	10.51	110	1.40	154	2.43
11B	11.40	120	1.22	147	2.51
12B	12.57	123	1.19	146	2.87
13B	14.68	123	1.22	151	3.09

[a]Each group contained 100 fish, except groups 2B and 12B, which contained 78 fish.
[b]Median Survival Time.
[c]Based on a measurement of total diet consumption throughout the experiment and an estimation of mean fish weight at the MST. Estimation of the latter assumes a constant rate of fish growth between average initial and final fish weights.
[d]Approximate total DDT intake up to MST in mg/kg.

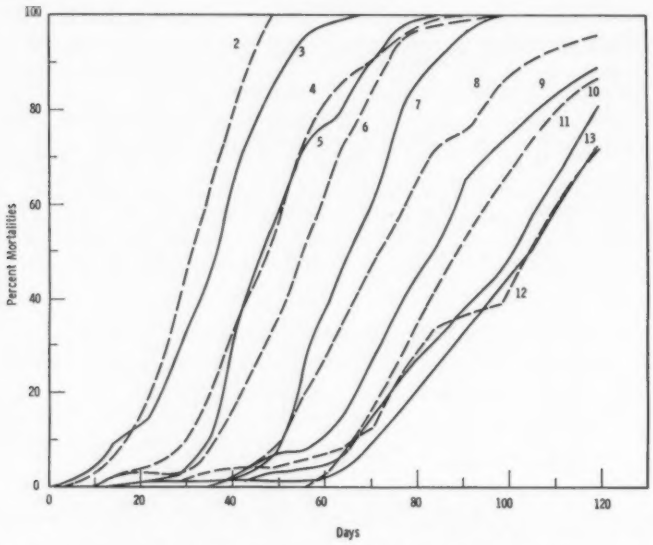

FIG. 1. Cumulative percent mortalities with respect to time in groups of selected coho salmon of uniform weight that were fed to satiation a diet containing 200 ppm technical DDT. Weight ranges of test fish were: group 2A (2.00–2.99 g); 3A (3.00–3.99 g); 4A (4.00–4.99 g); 5A (5.00–5.99 g); 6A (6.00–6.99 g); 7A (7.00–7.99 g); 8A (8.00–8.99 g); 9A (9.00–9.99 g); 10A (10.00–10.99 g); 11A (11.00–11.99 g); 12A (12.00–12.99 g); and 13A (greater than 13.00 g).

ability plot of cumulative mortalities versus time for these fish showed a typical time-percent effect type relation (Litchfield, 1946), forming a family of lines of almost parallel slope (Fig. 2). Larger fish were less susceptible to the toxic diet than were the smaller, and the Median Survival Time (MST, time in days necessary for one-half of the fish to die) appeared to be directly proportional to the initial weight of the fish (Table 2, Fig. 3).

Although diet consumption and, therefore, total DDT ingested per fish, increased with size, DDT intake per unit weight of fish declined (Table 2, Fig. 4). Initially, the daily amount of pesticide ingested per unit of body weight was an inverse function of the size of the fish but DDT intake per unit body weight eventually leveled off because the larger fish tended to consume somewhat greater quantities of diet (Fig. 4). Since the dose of DDT in milligrams per kilogram determined the rapidity with which the young salmon were poisoned, the daily oral dose of DDT per kilogram body weight was, thus, inversely proportional to the MST (Fig. 5). Young salmon apparently found the DDT diet to be somewhat unpalatable since intake of the pesticide ration was significantly reduced from that of fish receiving only the basal diet.

When the daily intake of DDT by salmon selected for equal body size was initially fixed through controlled feeding and the fish also supplemented with diet free of pesticide (B groups), mortalities occurred at a reduced rate

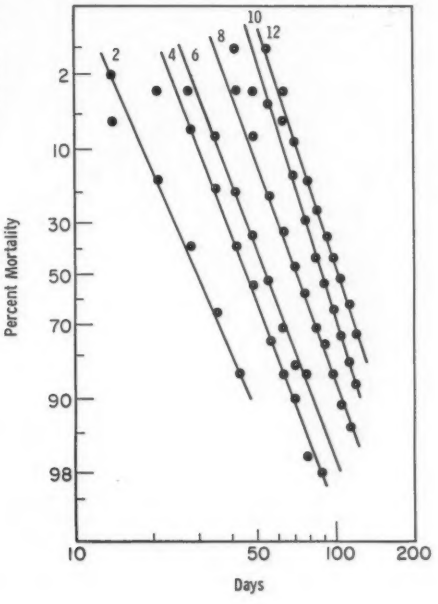

FIG. 2. Logarithm-probability plot of cumulative percent mortalities versus time for groups of selected coho salmon of uniform weight fed to satiation a diet containing 200 ppm technical DDT (A groups). Data on groups 2A, 4A, 6A, 8A, 10A, and 12A only are presented.

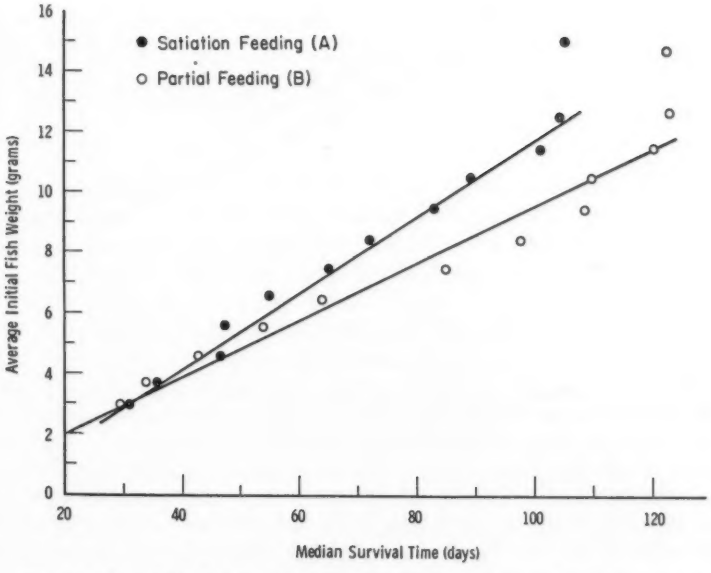

FIG. 3. Plot of Median Survival Time versus initial fish weight with groups of selected coho salmon of uniform weight receiving technical DDT in the diet.

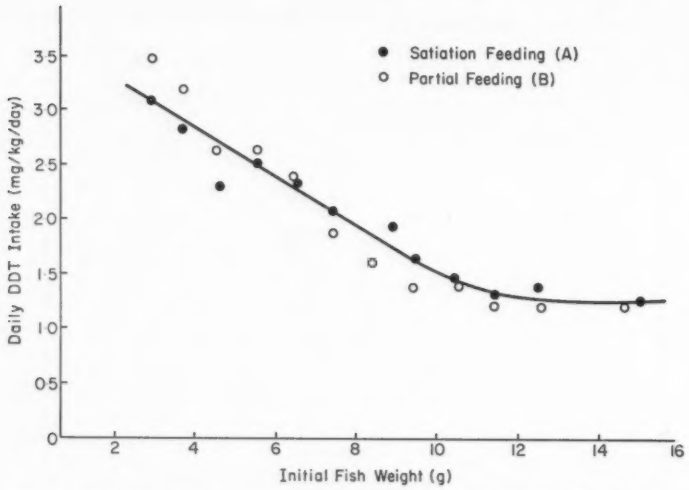

FIG. 4. Relation between initial fish weight and the daily DDT intake (mg/kg per day) for groups of selected coho salmon of uniform weight fed a diet containing technical DDT.

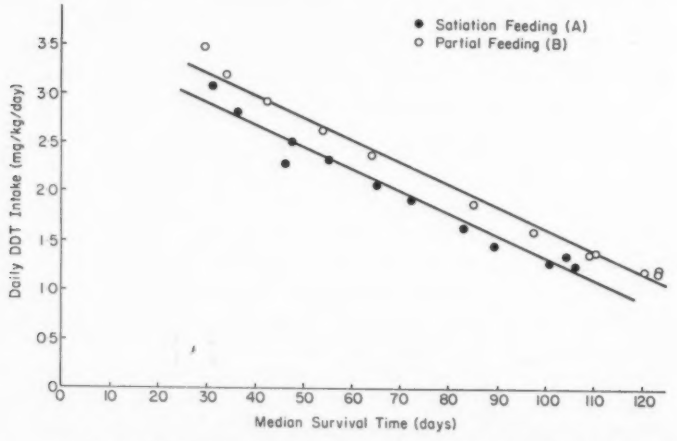

FIG. 5. Plot of Median Survival Time versus daily DDT intake (mg/kg per day) with groups of selected coho salmon of uniform weight receiving a diet containing technical DDT.

(Fig. 6). However, the smaller fish on this regimen also succumbed more readily to DDT than did the larger fish and MST increased in direct proportion to fish weight (Table 2, Fig. 3). Pesticide-fed fish that were supplemented with control ration (B fish) lived longer than fish of comparable size fed to satiation on DDT diet alone as shown by the reduced slope of the MST body weight

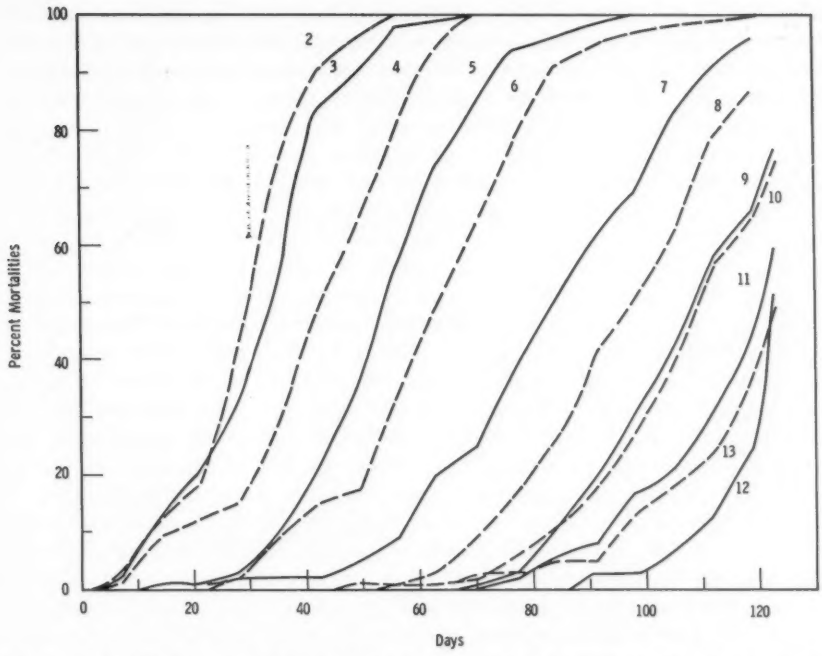

FIG. 6. Cumulative percent mortalities with respect to time in groups of selected coho salmon of uniform weight that were fed a diet containing 200 ppm technical DDT supplemented with feeding nonpesticide diet. Initial weight range of test fish was: group 2B (2.00–2.99 g); 3B (3.00–3.99 g); 4B (4.00–4.99 g); 5B (5.00–5.99 g); 6B (6.00–6.99 g); 7B (7.00–7.99 g); 8B (8.00–8.99 g); 9B (9.00–9.99 g); 10B (10.00–10.99 g); 11B (11.00–11.99 g); 12B (12.00–12.99 g); 13B (greater than 13.00 g).

curve in Fig. 3. Although the B fish initially consumed less DDT per fish on a daily basis, control diet supplementation apparently helped to maintain appetite for the pesticide ration and the fish eventually consumed equal or greater quantities of DDT per kilogram of body weight (Table 3) than fish that received the DDT ration alone (Table 2).

The cumulative 50% lethal dose (cumulative LD_{50}, dose of DDT in milligrams per kilogram body weight ingested during the time required to kill one-half of the test fish) provided a measure of the relative sensitivity of salmon to the pesticide. Although the amount of toxicant fed is not necessarily identical to the amount absorbed or retained by the fish, previous studies (Buhler et al., 1969) suggest that almost all of the DDT consumed in the diet is assimilated by coho salmon and that very little of the absorbed pesticide subsequently was excreted by the fish. Hence, the pesticide dose received by the fish can be estimated from the diet consumption data.

Fish weighing between 2 and 7 g and fed to satiation (A groups) were more susceptible to a given cumulative LD_{50} dose of DDT than were larger

fish. The 2–3-g fish exhibited the lowest cumulative LD_{50} and this dose then increased in direct proportion to the body weight for fish up to the 7-g size (Table 2, Fig. 7). The cumulative lethal dose, however, was found to be constant and essentially independent of body weight for salmon that initially weighed more than 7 g (Fig. 7).

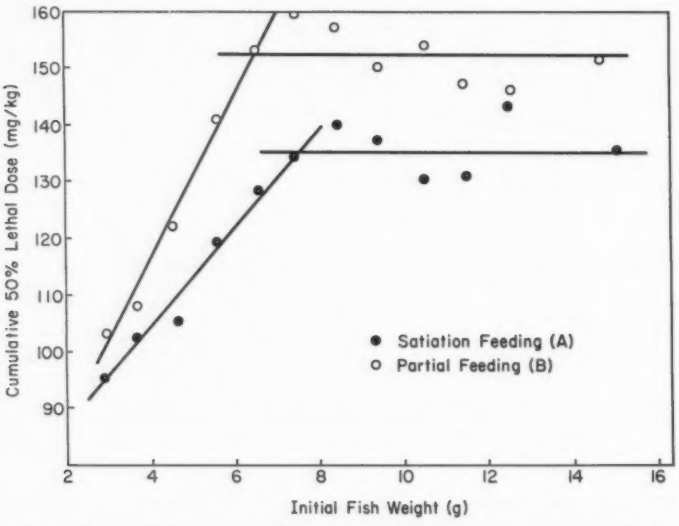

FIG. 7. Relation between initial fish weight and the cumulative 50% lethal dose for young coho salmon fed a diet containing technical DDT.

Salmon fed a partial DDT diet supplemented with pesticide-free ration (B fish) were less affected by the pesticide as shown by the upper plot in Fig. 7. The smallest fish on this regimen were also most sensitive to DDT and the cumulative LD_{50} increased in proportion to body weight up to the 7-g size (Table 3, Fig. 7). Fish that initially weighed more than 7 g, however, tended to accumulate approximately the same lethal dose (Fig. 7).

DISCUSSION

Sensitivity to chemicals varies with the developmental state of the fish since eggs and larva are known to be much more resistant to pesticides (Iyatami et al., 1958) or to fluoride (Neuhold and Sigler, 1960) than are the fry or adult animals. After the yolk-fry or larval stage, tolerance to toxic chemicals is reduced but then subsequently increases with fish age and size.

The influence of age or body size on the sensitivity of fish to toxicants has been considered by several investigators although in most studies no attempt has been made to distinguish between these two factors. For example, low concentrations of toxaphene can selectively kill the small fish in a lake

(Fukano and Hooper, 1958). Holden (1964) has reported that brown trout (*S. trutta*) fry were 25 times more sensitive to DDT than were fish several months old. Weiss and Botts (1957) observed that larger fish were more tolerant to the organic phosphorus compound Sarin than were smaller fish. In acute toxicity studies with pesticides (Mount, 1962) and chemical control agents (Surber, 1948) tolerance was observed to increase with the size of the fish. Pickering et al. (1962) investigated the influence of age on mortality in some detail and reported that the TL_m values for organic phosphorus insecticides decreased with age in fathead minnows (*Pimephales promelas*). Neuhold and Sigler (1960) noted that the MST for carp (*Cyprinus carpio*) and rainbow trout exposed to fluoride increased as a function of increasing fish size. Goodman (1951) found that rainbow trout were less susceptible to solutions of zinc as they grew older. Burdick et al. (1965) observed that the toxicity of the carbamate insecticide Sevin to brown trout decreased with increasing length.

On the other hand, no significant effect in susceptibility of different sized fish to inorganic and organic toxicants was found by Cairns and Scheier (1958) or for various petrochemicals studied by Pickering and Henderson (1966).

Recent experiments with coho and chinook salmon (Buhler et al., 1969) have shown that younger fish were more susceptible to dietary DDT than were the larger older fish. There was also a selective loss of the smaller fish from exposed populations of the same age containing fish of varying sizes. In chronic studies with normal populations of identical age cutthroat trout, Allison et al. (1963, 1964) also noted higher mortalities among the smaller slower growing fish after either oral or immersion exposure to DDT.

Body size and weight have now been shown to be major factors affecting the relative susceptibility of young salmon to DDT. The MST for coho salmon of equivalent age exposed chronically to dietary DDT is directly proportional to the initial weight of the fish (Fig. 3).

Body size influenced oral toxicity primarily by controlling the total diet consumption per fish and, hence, the milligrams per kilogram dose of DDT. As has been shown repeatedly, the rates of metabolism of animals including fishes are inversely proportional to their body size (Winberg, 1960). As size increases, therefore, energy expended per unit body weight decreased in a regular manner and food intake by the fish also decreased proportionally (Brown, 1957). Inclusion of a toxicant such as DDT in the diet results in the smallest fish ingesting a proportionally greater milligram per kilogram dose of pesticide (Fig. 4) and, consequently, these fish are the first to become intoxicated.

In addition to controlling the dietary intake of pesticide by the fish, body size also appears to influence the relative susceptibility of young salmon to a given dose of pesticide. This conclusion follows from observations that the smallest salmon in the present experiment succumbed to a much lower cumulative lethal dose of DDT than did the larger fish (Fig. 7). The cumulative LD_{50} for DDT in the 2–7-g salmon increased in a linear manner with body weight, indicating that these fish develop a size-related tolerance to the pesticide.

Such results suggest that the smallest salmon may be unable to effectively store or metabolize absorbed DDT and that resistance to DDT gradually appears with increasing body size. Storage rather than metabolism appears to be responsible for the relative susceptibility of young coho salmon to DDT since these fish excrete absorbed DDT only very slowly and also tend to accumulate a more or less constant ratio of DDT and its metabolites in tissues with respect to storage time and body size (Buhler et al., 1969). This conclusion is supported by the results of studies with coho salmon (Buhler et al., 1969) and with lake trout, *Salvelinus namaycush* (Reinert, 1969), which have shown that the total concentration of DDT and metabolites stored in the body increases with the size of the fish. Greater detoxification of pesticide by storage probably is made possible by the trend towards a higher percentage of body lipids as fish size increases (Phillips et al., 1960; Vanstone and Markert, 1968; Reinert, 1969). A linear increase in the cumulative LD_{50} occurs with body weight (Fig. 7) in the 2–7-g salmon apparently because the total lipid content of these salmon increases more rapidly than does the body weight (Vanstone and Markert, 1968). A similar linear increase in the acute LD_{50} with body size has been noted by Bergmann et al. (1963) for the toxic substance ichthyotoxin in the minnow *Gambusia affinis*. These latter workers speculated that in this instance the linear relationship may derive from the relatively larger size of the brain in the juvenile minnow as compared with the adult.

The size of salmon initially weighing more than 7 g, however, has no apparent influence on the sensitivity of the fish to a given dose of pesticide (Fig. 7). The absence of a size-related effect on sensitivity in the larger salmon may result from a leveling off in the lipid content of these fish in a manner analogous to that observed with other hatchery-raised coho salmon (Vanstone and Markert, 1968). These investigators had noted that the rate of accumulation of body lipid changes significantly after the fish has reached about 10 g in weight. Under such circumstances the percentage of body lipid remains relatively unchanged as body weight increases further. Pesticide stored in the lipids would be directly proportional to body weight and the milligram per kilogram cumulative lethal dose of DDT then would be constant and independent of body size. Similarly, the acute LD_{50} dose, a measure of relative sensitivity, for animals is usually independent of the influence of body size for most chemicals (Lamanna and Hart, 1968).

The present experiments with coho salmon populations selected according to body size demonstrate that the smallest fish are more susceptible to dietary DDT. However, native fish populations normally contain a distribution of sizes since there is an order of dominance among fish and certain fish tend to be more aggressive than others so that they are able to successfully compete for the available food and grow more rapidly (Brown, 1957). Although competition for food in the presence of such a size hierarchy would reduce somewhat the individual intake of pesticides in the diet by each fish in the population, body size would still play a significant role in controlling appetite and hence relative pesticide ingestion as well as affecting the sensitivity of the fish to

the toxicant. Such size-related differences in response to toxic chemicals may be even more pronounced when the population density is relatively low or the availability of food is very great. Such factors can result in a differential mortality that deprives a given year-class of fish of the smallest members of the group (Allison et al., 1963, 1964). Under conditions where there is a more or less continuous input of pesticides into the food chain, a selective annual mortality of the smallest fish in a wild population would materially reduce the chances for survival of that population.

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A Theory Concerning the Distance Travelled by Animals Entering the Drift of a Stream

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MCLAY, C. 1970. A theory concerning the distance travelled by animals entering the drift of a stream. J. Fish. Res. Bd. Canada 27: 359–370.

Stream bottom animals were introduced into a stream by disturbing the gravel at increasing distances upstream from a drift sampler. The relationship between the number of animals arriving at the sampler and distance of origin of the animals was found to be $N_x = N_0 e^{-RX}$ where N_0 is the number of animals introduced, R is the rate of return to the gravel of the animals, and X is the distance upstream from the sampler. The maximum distance travelled was 45.7 m and the mean distance 10.7 m. The values of R for individual species ranged from 0.0517 to 1.962 and values of the mean distance travelled from 19.3 to 0.5 m. Specific differences could be accounted for by differences in structure or behaviour of the animals.

From these observations, a model of stream drift is developed. Two previously published experiments are shown to conform to model relationship: (1) recovery of drift density downstream from a blockage in the experiment of Waters (Ecology 46: 327–334, 1965); and (2) distribution of times taken to regain a foothold on the substrate by mayfly nymphs released into a current (Madsen, Flora Fauna 72: 148–154, 1966; Hydrobiologia 31: 337–349, 1968).

Since a stationary feeding fish such as a trout can be considered to be a "drift sampler" intercepting particles in the current, some suggestions are made about the development of a theory concerning the delivery of items of food to such a predator.

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INTRODUCTION

MOST ANIMALS that occur in the bottom fauna of a stream also occur in the drift. Moreover, the proportional occurrence of species in the drift is generally very similar to that in the bottom fauna. Where it is not, deviations can be accounted for in terms of behaviour or structure of the animals (McLay, 1968). When an animal drifts, it becomes relocated in some other part of the substrate. Therefore, most of the members of the fauna of any small part of the substrate are probably recent arrivals from surrounding areas. Animals may immigrate from the immediately surrounding area (Moon, 1940) and from both upstream and downstream directions (Waters, 1964), but in both instances the distances involved are unknown.

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This paper reports an experiment that was planned to determine how far downstream animals would drift, and thus conversely to suggest where immigrants to an area might come from.

Animals in the drift are not only a source of immigrants to a small area of substrate, but can be eaten by predators such as fish. The results of this experiment and some previously published information, suggest possible development of a theory concerning delivery of food items to a stationary feeding animal, such as a trout, by a current of water.

METHOD

The method, in essence, consisted of introducing animals into the current at increasing distances upstream from a sampling point where their occurrence in the drift was to be detected. Since drift in a stream has been shown to be a continuous phenomenon, it was necessary to assume that normal drift continued throughout the experiment and therefore the occurrence of introduced animals was to be detected against the background drift. The experiment was done in the daytime, when the number of animals in the drift is much smaller and more constant than at night.

The experiment was conducted in the Silverstream, a small tributary of the Tairei River near Dunedin, New Zealand, on April 4, 1965, and between 0900 and 1600 hr under conditions of bright sunshine. The water temperature was approximately 10 C during the experiment. The section of stream used was straight, with uniform gradient, and uniform coarse gravel substrate, 36 m long with mean width 6–7 m, mean depth 15–20 cm, and mean current velocity 21 cm/sec. There were no emergent objects in the stream that could have disrupted the flow, channelling the water to one or the other bank.

A drift sampler, based on the design published by Cushing (1964) (for description see McLay, 1968), was placed in midstream, and markers were placed in the bank at intervals of 6 m above the sampler, up to 36 m. The procedure used was to first take a half-hour control drift sample. Then a pulse of animals was introduced into the current by disturbing an area of the stream bottom approximately 20 cm wide across the width of the stream, and a second half-hour sample was collected. The substrate was disturbed in the same manner as for taking a Surber sample and took approximately 2 min. The experimental sample was timed from the beginning of the disturbance. The experimental procedure was first done at the 6-m mark and then was repeated at increasing intervals upstream with a control period preceding each experimental period. None of the animals obtained in the drift was found to be damaged in any way attributable to the effects of the disturbance.

The net drift density[2] (N) resulting from the introduction, was obtained by subtracting the control drift density (C) measured immediately prior to introduction from the experimental drift density (E). It was assumed that the magnitude of each pulse of animals introduced into the current was equal. Since the technique of introduction was the same at each distance, the assumption is that the density of the animals did not show any trend along the length of the section of stream. Since the rate at which the pulse of animals would arrive at the sampler was unknown, the comparatively long sample period of 0.5 hr was chosen: a period longer than if the pulse travelled as a unit at the velocity of the stream. As the distance of the point of introduction from the sampler increased, so the time required for the pulse of animals to reach the sampler also increased. No correction was applied to the volume of water filtered which takes this into account because even at the 36-m mark this delay constituted less than 10% of the sample period. The time remaining was apparently adequate to collect a sample of the pulse of animals

[2]In a previous paper (McLay, 1968) the term "drift rate" was used to refer to the numbers of animals per unit volume of water filtered by the drift sampler. Here the term "drift density" is used, following the distinction made by Waters (1969).

because the control drift densities between experimental periods showed no tendency to increase (Table 1). Furthermore, the experimental samples contained all members of the bottom fauna whereas the control samples contained only Chironomidae larvae, *Oxyethira albiceps* larvae, *Pycnocentrodes sp.* larvae, and the occasional *Deleatidium sp.* and *Austroperla cyrene* nymphs.

TABLE 1. Effects on drift density of disturbance of bottom fauna at increasing distances from the sampler. All drift densities are in numbers/m^3.

Distance from sampler (*m*)	Exptl. drift density	Control drift density	Net drift density
6	57.1	4.0	53.1
12	32.4	6.5	25.9
18	23.4	6.0	17.4
24	20.7	7.8	12.9
30	14.4	7.8	6.6
36	9.6	7.2	2.4

RESULTS

As the distance upstream of the disturbance increased, the number of animals that drifted to the sampler decreased (Table 1). The control drift density was relatively constant between 4.0 and 7.8 organisms/m^3. The experimental drift density ranged from 57.1 from 6 m upstream, to 9.6 from 36 m. The relation between net drift density and distance was a hollow curve (Fig. 1A) that was apparently linear in a plot of log net drift density against distance (Fig. 1B). This implies that the animals once disturbed, tend to go out of the drift at a constant instantaneous rate. Thus, the change in numbers of animals with distance (x) is given by:

$$\frac{dN}{dx} = -RN$$

or, in integral form,

$$N_x = N_0 \, e^{-RX}$$

and, in straight line form,

$$\ln N_x = \ln N_0 - RX$$

where R is the instantaneous rate of disappearance from the drift, N_x is the number in the drift at X distance from the disturbance, and N_0 is the initial number of animals.[3] For the whole group of drift animals in the experiment,

[3]Since the dimensions of the intake of the sampler were 2 cm wide and 10 cm deep, then N_0 is the number of animals in a plane whose area is 20 cm^2 and this plane can be imagined as being located at the end of a "column" of water whose length is (current velocity times length of sample period), i.e., (0.21 · 1800) m = 378. The animals came from an area of gravel of dimensions 2 cm wide by 20 cm long, and since the depth of the water was 20 cm, they were introduced into the plane without dilution.

N_0 = 93.78 and R = 0.09371 in the least-squares fit of the regression of ln N_0 on X.

The shape of the relationship in Fig. 1 is not dictated by the occurrences of a few species. Individually, most of the species showed the same general form of relationship between number of animals arriving at the sampler and

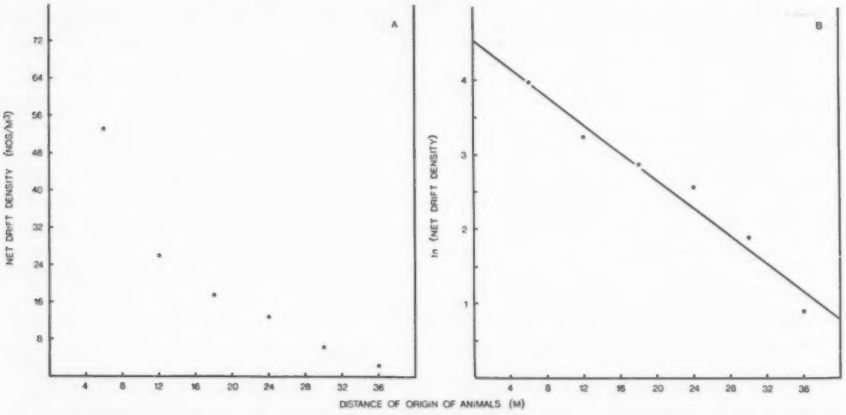

FIG. 1. (A) Values of the net drift density as a function of distance from the sampler of the origin of the animals. (B) Demonstrating the linear nature of the relationship between ln N_x and distance from the sampler. Equation of the line is ln N_x = 4.541 − 0.09371 X, the correlation coefficient r = 0.9837.

distance (Fig. 2). The values of R, shown for some of the species in Table 2, reflect differences in the rate at which various kinds of organisms settle out of the drift back into the substrate. They range from 0.0517 for the Rhyacophilidae larvae to 1.962 for *Olinga*, suggesting quite substantial differences between species, which seem to be largely related to differences in structure, size, and behaviour. For example, *Oxyethira* is one of the very small Trichoptera larvae with a transparent, purse-shaped case and seems to be incapable of any kind of swimming. Chironomidae larvae are similarly small and are capable of movement by a violent wriggling, but many of the recorded animals were encased in algae. Nymphs of the mayfly *Deleatidium sp.* are small, flattened, and able to swim very vigorously and yet the Elmidae larvae, which are small, cylindrical nonswimmers, had a similar value of R. The swimming ability of the mayfly does not, therefore, seem to result in a more rapid return to the substrate. The larvae of *Pycnocentrodes* sp. are contained in small cylindrical cases constructed of fine sand and are incapable of swimming. The density of their cases would be expected to cause the observed rapid return to the gravel. All individuals of *Olinga* in the sample were large and, since the larvae are each inside a heavy horny case, they would not be expected to remain long in the drift. Since they were recorded only from the first introduction at 6 m the relationship of Fig. 2G is probably spurious. The best fit was obtained with

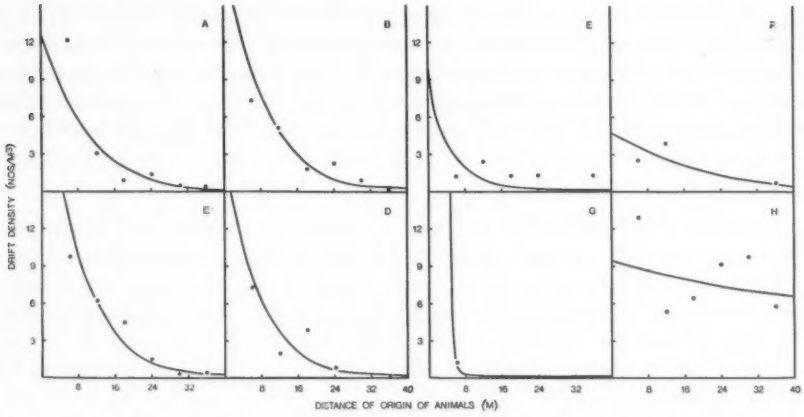

FIG. 2. Relationship between the drift density and distance for some species. The correlation coefficient for each relationship is given after the name. A, *Oxyethira albiceps* larvae (0.9304); B, Chironomidae larvae (0.9166); C, *Deleatidium* sp. nymphs (0.9561); D, Elmidae larvae (0.6319); E, *Pycnocentrodes* sp. larvae (0.4104); F, Rhyacophilidae larvae (0.9139); G, *Olinga feredayi* larvae (0.999); H, other species taken together (0.2693).

TABLE 2. Values of rate of return to the substrate (R) and mean distance (1/R) travelled by the animals.

Species	R	$\bar{x}$ (*m*)
Rhyacophilidae larvae (Trichoptera)	0.0517	19.3
Oxyethira albiceps larvae (Trichoptera)	0.1024	9.8
Chironomidae larvae (Diptera)	0.1257	8.0
Deleatidium sp. nymphs (Ephemeroptera)	0.1257	8.0
Elmidae larvae (Coleoptera)	0.1366	7.3
Pycnocentrodes sp. larvae (Trichoptera)	0.1781	5.6
Olinga feredayi larvae (Trichoptera)	1.962	0.5
"Other members of bottom fauna" taken together	0.0084	119.5
"All members of the bottom fauna" taken together	0.09371	10.7

a very steep line whose *y* intercept (ln N_0) was enormous, suggesting that there were very large numbers of *Olinga* in the bottom fauna. Since this animal is not very common the slope has undoubtedly been overestimated, but without further data this cannot be corrected. Rhyacophilidae larvae are large, long-bodied, uncased Trichoptera that are capable of strong movements resulting from contractile flickings of the whole body. Although they were capable of very rapid return to the substrate, individuals were recorded from as far as 36 m. The very low value of R for their relationship is inexplicable in terms of their structure or known behaviour. Between introduction and arrival at the sampler they must have made contact with the substrate many times. The remaining species, which included small Oligochaetes, some Plecoptera

nymphs, and *Oxyethira* pupae, have all been combined and a value of R of 0.0084 was obtained. This value is, of course, inherently meaningless because it applies to an aggregate of species, but it is considerably smaller than those listed in Table 2 and thus explains why R for all bottom fauna species t ken together was only 0.09371 whereas most other values of R were larger.

A Model of Stream Drift

Because the relationship between numbers of animals and distance is semi-logarithmic, the simple model is suggested that animals return to the substrate at a constant instantaneous rate. The process of settling over distance is apparently analogous to mortality in time. More formally, if N_0 organisms in a strip of stream substrate of some unit width are disturbed, they subsequently "settle" from the drift at a rate that is a constant proportion of the number remaining, i.e.,

$$\frac{dN}{dX} = -RN$$

and in integral form, the number of animals at a point X from the site of disturbance (N_x) is given by

$$N_x = N_0 e^{-RX}. \tag{1}$$

The mean distance travelled by an organism is given by the sum of the distances travelled by the individual organisms, divided by the total number of organisms, viz:

$$\bar{x} = \int_0^\infty \frac{N_0 e^{-RX} dX}{N_0} = \frac{1}{R}.$$

Values of the mean distance travelled in the experimental samples are given in Table 2, and for all animals the mean distance was 10.67 m.

The maximum distance an organism could travel is infinity if there was an infinite number to begin with (N_0). More realistically, the distance to which, say, 1% of the animals travel ($X_{.01}$) may be estimated directly by substituting .01 for the ratio $\frac{N_x}{N_0}$ in equation 1 and rearranging, i.e.,

$$.01 = \frac{N_x}{N_0} = e^{-RX}$$

or

$$X_{.01} = \frac{-\ln\left(\frac{N_0}{N_x}\right)}{R} = \frac{\ln (100)}{R}.$$

For experimental samples of Table 2, the distance at which 1% of the animals are still in the drift is 49.2 m. The number of animals introduced by disturbance at a source x meters upstream from a fixed point arriving at that point is given by equation 1. Then, provided that the contributions were strictly additive, if two or more pulses of animals were to arrive from different distances, the total number of animals (N_T) that would be caught would be given by

$$N_T = N_0 e^{-RX}1 + N_0 e^{-RX}2 + \ldots\ldots + N_0 e^{-RX}n$$

where n is the number of sources. Then, if the number contributed by each source were the same and animals arrived from all possible points upstream for a distance x, the total number caught would be given by

$$N_T = \int_0^x N_x \, dx = \int_0^x N_0 e^{-RX} \, dx = \frac{N_0}{R}[1 - e^{-RX}]. \qquad (2)$$

The total number of animals that would be taken may be considered as coming potentially from an infinite distance upstream. As X approaches infinity, N_T approaches $\frac{N_0}{R}$. Thus, for any stream system, an experiment like the foregoing enables an estimate of potential drift from simultaneous disturbance of the whole bed of the stream. In the particular case of the experiment here reported, maximum N_T (N_{max}) is almost precisely 1000. The relationship of equation 2 is shown in Fig. 3. At large distances from the sampler (i.e., in excess of 50 m) the disturbance has little effect on the total number of animals taken in the sampler because most of the animals originating from these distances have already returned to the gravel.

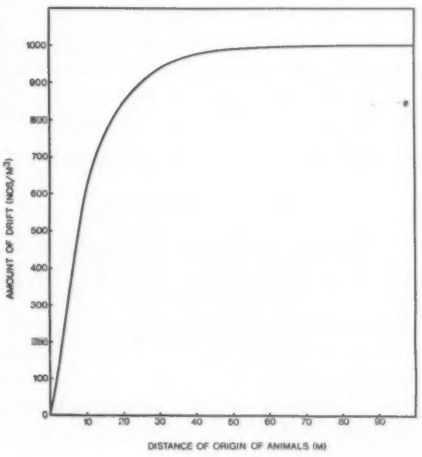

FIG. 3. The amount of drift that would be delivered to a drift sampler if the gravel had been simultaneously disturbed at all distances from zero to some distance upstream. The limiting value for the drift rate is C/R = 1000.74.

No experiments were performed in which simultaneous disturbance of the bottom fauna at a number of points was produced. Therefore, the predicted relationship between N_T and X could not be tested. However, the case of simultaneous "disturbance" over a length of stream producing drift at some point in that length is one way of interpreting naturally occurring drift. If the natural drift were interrupted at any point in a stream, the manner in which drift increases with increasing distance downstream from the interruption should be as shown in Fig. 3. The increase is initially very fast because fewer animals are returning to the bottom than are entering the drift, but the rapidity of the increase soon decreases as the rate of these two processes becomes similar and the derivative of the relationship approaches zero.

Such a blockage experiment has been performed by Waters (1965). All drift into a length of stream was prevented by filtering all the water in the stream through a set of drift nets. Then at increasing intervals downstream from the blockage, the number of animals in the drift was sampled between 1900 and 0230 hr. As controls, the drift of each station was sampled on the previous night without the block nets in position. The numbers obtained with the block nets in position were expressed as a percentage of the control for each station.[4] These results are shown in Fig. 4 for *Baetis* and *Gammarus*.

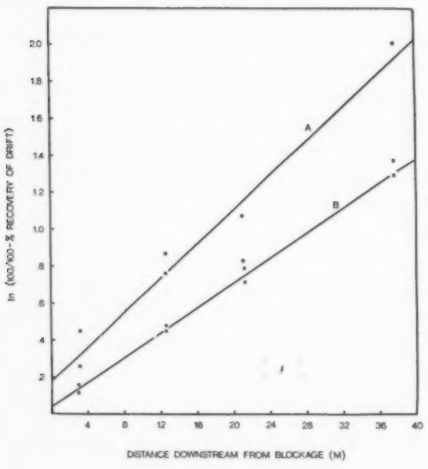

FIG. 4. Percentage recovery of the drift as a function of the distance downstream from the blockage; A, *Baetis vagans* (solid circles); B, *Gammarus limnaeus* (open circles). Correlation coefficients for the relationships are 0.9702 and 0.9981 respectively (data from Waters, 1965).

Waters' results are expressed as the percentage of normal drift at various distances downstream from the blockage. Rearranging equation 2, denoting $\frac{N_0}{R}$ as N_{max}, and taking logarithms, we have

[4]It should be noted that because the recovery of the drift is expressed as a proportion of the normal level, it is not necessary to fulfil the requirement of having a large length of stream upstream from the block nets.

$$\ln\left(\frac{N_{max}}{N_{max} - N_T}\right) = RX$$

and if N_T is expressed as a percentage of N_{max},

$$\ln 100\left(\frac{N_{max}}{N_{max} - N_T}\right) = RX \qquad (3)$$

which is linear through the origin. It is to be noted that $N_{max} = \frac{N_0}{R}$ so that R appears on both sides of equation 3. To solve for R and N_{max} by least squares requires an iterative procedure similar to that of Allen (1966), the form of equation 3 being similar to the von Bertalanffy growth curve. However, for the data of Waters (1965), an estimate of N_{max} is available from the measurement of "normal drift," so equation 3 reduces to

$$\ln\left(\frac{100}{100 - Q}\right) = RX \qquad (4)$$

where Q is N_T expressed as a percentage of N_{max} (i.e., $Q = 100\frac{N_T}{N_{max}}$).

Fitting Waters' data by the usual method of least squares, R is estimated as 0.04641 for *Baetis* and 0.03505 for *Gammarus*, and the *y* intercepts are both close to zero (0.1748 and 0.0261 respectively). The predicted form of the relationship gives an excellent description of the data, but no samples were taken at distances where the drift had fully recovered from the effects of the blockage. Extrapolation of the relationships in Fig. 4 suggests that this distance should have been 99.2 m for *Baetis* and 131.4 m for *Gammarus*. It seems that Waters (1965) assumed a simple linear model for the relationship since he estimated that these distances should lie in the range 50–60 m. The mean individual distance travelled (1/R) was 21.6 m for *Baetis* and 28.5 m for *Gammarus*. The values of R for both *Baetis* and *Gammarus* are exceptionally low when compared with those obtained in the disturbance experiment. The explanation is perhaps that Waters' observations were at night when drift might be further, and his stream had higher current velocity (approximately 1 m/sec as opposed to 21 cm/sec).

A second series of experiments adequately described by the model are those of Madsen (1966, 1968). Mayfly nymphs (*Baetis* sp. *Ephemerella ignita*, *Heptagenia sulphurea*, and *H. fuscogrisea*) were individually released into a long tank with current velocity of 20–30 cm/sec and measurements were made of the time taken for each animal to attain a firm hold on the substrate. Assuming that there is a direct relationship between the times measured and the distance travelled, Madsen's observations can be interpreted as the distances at which various numbers of animals were removed from the drift by settling on the bottom. The remainder, those still in the drift at a certain distance,

expressed as a percentage of the number put in, would thus be predicted by equation 1, i.e.,

$$N_x = N_0 \, e^{-RT}$$

$$\ln \left(100 \frac{N_x}{N_0}\right) = \ln 100 - RT,$$

which is linear with intercept ln 100. Figure 5 gives some of Madsen's observations plotted as appropriate for equation 1, the expectation being a straight line with intercept ln 100%. In each instance the fit to a straight line is close, and in none of the six instances is the correlation coefficient less than 0.94. The intercepts depart substantially from ln 100, in all instances suggesting an initial value of between 58 and 72%. This departure suggests that

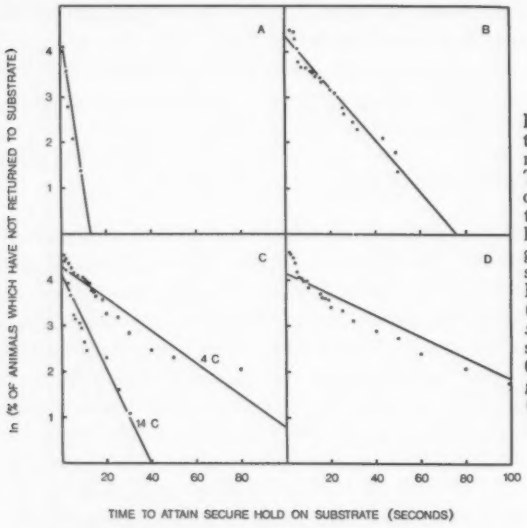

FIG. 5. The distribution of times taken for four species of mayfly nymphs to return to the substrate. The natural logarithm of percentage of observations is plotted against the time taken. The slope of the line and correlation coefficient are given after each name. A, *Baetis* sp. at 11 C (–0.3302, 0.9528); B, *Ephemerella ignita* at 11 C (–0.05633, 0.9807); C, *Heptagenia suphurea* at 14 C (–0.1016, 0.9636), solid circles, and at 4 C (–0.03487, 0.9421), open circles; D, *H. fuscogrisea* at 14 C (–0.02276, 0.9450) (data from Madsen 1966 and 1968).

the rate of settling at short distances is greater than expected from equation 1 or that the rate of settling is lower than expected at great distances, or both. However, the 95% confidence limits of the intercept in all instances but one include the theoretical intercept of ln 100%, and in the one exception, the limit extends to ln 97%. The fit to the model is adequate. An interesting and unsuspected effect of water temperature on *H. sulphurea* is shown in Fig. 5C. The nymphs took longer to return to the substrate at 4 C than they did at 14 C, at all times after release the proportion that had not returned to the substrate was larger at 4 C.

DISCUSSION

There seem to be two attributes of an animal that determine how far it will be transported when it enters stream drift: (1) the physical properties

of the animal — its size, shape, density, centre of gravity; and (2) the behaviour — the kind and amount of "swimming" and the changes in shape the animal might perform. Every animal has values for the first class of properties but only some perform actively. Additionally, any of their reactions may change with stages of the life history. Presumably, if all reacted in the same manner, then all would spend the same time returning to the substrate. But, as the various patterns of Fig. 5 show, the reactions of the various animals are not the same. Madsen (1968) notes that the reactions of the mayfly nymphs that he used vary from being almost completely passive to active swimming towards the substrate or even against the direction of current flow. Under natural conditions in a stream there is turbulence and an animal's first contact with the substrate may not be the point at which it stops. Many animals may have to be exposed to a number of trials before successful attainment of a foothold. It should be noted that the reactions of the animals disturbed from the substrate in the disturbance experiment may have been slightly different if they had entered the drift in a natural manner. However, there is no reason to doubt that the relationship between distance downstream from the source and proportion of animals returning to the substrate should be of any different form. Also all the animals listed in Table 2 were found to occur normally in the drift and so could be expected to be able to return to the gravel.

Despite these many variables, it would appear that for individual species as well as for drift in the aggregate the model here developed has some generality. Natural drift at any point in a stream may be considered as the sum of a large number of contributions each from some distance upstream, each contribution depending upon the number of animals entering the drift at that point (N_0), its distance from the reference point, and the rate at which animals return to the substrate. The drift density at any point in the stream is, then, the result of many events occurring both at some distance upstream and at a time prior to the time of the observation at that point.

In the present experiment the value of N_0 was the result of disturbance of the substrate. What determines this quantity under natural conditions has been the subject of much discussion, which has been summarized by Waters (1965) and Ulfstrand (1968). Some species only occur in the drift at night (McLay, 1968) and others mainly during the day (Waters, 1968). Thus, light intensity limits the time of occurrence of some species. It is not at all certain that N_0 is density dependent. A mechanism involving physical contact that results in entry of some animals into the drift has been proposed by McLay (1968). For some species N_0 may be constant, provided that numbers in the gravel do not change greatly, since their appearance in the drift seems to be the result of being accidentally swept away by the current.

The absence of information about a number of quantities and relationships prevents development of a satisfactory model of the complete picture of dispersal of stream animals. Some of these are the distance and direction moved while in the gravel, the frequency with which an individual occurs in the drift and the effect of the current velocity upon the rate of return to the substrate.

If such information were available, it would be possible to test a theory concerning the delivery of food items to a feeding fish since, like a drift sampler, it can be regarded as an interceptor of a flow of particles. It would then be possible to show how a fish utilizing animals in the drift could be harvesting bottom fauna of an area of stream above it and, further, how interception of food items by a fish could affect the supply to others downstream.

The simplest model of stream dispersal would balance upstream movement through the gravel with downstream dispersal by drift, the animals moving down and then upstream in a cyclic manner. In this instance predation by fish would lead to depletion of the fauna within a restricted length of stream. Such counter-balanced dispersal may be the kind operating in the case of *Gammarus* (Minckley, 1964; Muller, 1954; Waters, 1962; and Hultin, 1968). But for some species there is probably some net downstream movement. In this instance, the effects of fish feeding might influence numbers of animals for many miles downstream. Further, if the value of R is inversely related to current velocity, the best position for a drift-feeding fish would be in a pool at the downstream end of a stream section with high current velocity.

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Growth and Chemical Composition of Chum (*Oncorhynchus keta*) and Sockeye (*O. nerka*) Salmon Fry Produced in Spawning Channel and Natural Environments

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A comparison was made of the weights, lengths, weight–length relations, and chemical composition in migrating chum and sockeye fry resulting from eggs incubated and hatched in natural and artificial streams. Additionally, growth rates and rates of change in some chemical constituents were investigated in postmigrant channel- and river-hatched chum salmon confined to floating pens in sea water and in postmigrant sockeye salmon captured from their lake nursery area.

There were no apparent differences in the lengths, weights, weight–length relations, or chemical composition between river- and channel-hatched chum salmon migrants. Nor was there any difference in the rates of change in length, weight, or chemical composition of these two groups of chums when confined to pens in sea water for 10 weeks after migration.

There were differences in the weight–length relations, lipid content, and nitrogen content between channel- and river-hatched sockeye migrants. It is suggested, however, that these differences are due to the fact that the timing of the peaks of migration of these two groups of fish differed and that at any one time channel fry were physiologically different from river fry.

Growth of the chum salmon in sea water and the sockeye in the lake was exponential and the slope of the weight–length relation, $W = aL^b$, was approximately 3.25. During this growth period although total weight, moisture, solids, lipid, and nitrogen increased the rate of increase in moisture was less than the rates of increase in total weight or the other constituents.

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INTRODUCTION

SPAWNING CHANNELS are being developed in British Columbia by the Department of Fisheries and Forestry of Canada for the purpose of increasing the production of indigenous stocks of Pacific salmon. Two of these channels have been constructed at Big Qualicum River on the east coast of Vancouver Island (Lister and Walker, 1966) and at Fulton River a tributary of Babine

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Lake (McDonald, 1969). One of the premises on which spawning channel construction is based is that there are no differences in the fry from the same parental stock but reared in natural and artificial streams. An opportunity to investigate one aspect of this basic premise occurred in 1966 when a comparison of the chemical composition of spawning channel and river chum salmon fry (*Oncorhynchus keta*) and sockeye salmon fry (*O. nerka*) from these two rivers was made. The results of this comparison, together with a comparison of the growth rates of captive channel and river chum fry in sea water and wild underyearling sockeye fry from Babine Lake, form the basis of the present communication.

MATERIALS AND METHODS

Chum fry were captured from the Big Qualicum River and the Big Qualicum River artificial spawning channel, at their enumeration sites about 1 mile above the river mouth, during the peak of their seaward migration from May 2 to May 18, 1966. On May 11 additional river and channel migrants were transported to pens placed in Deep Bay, 8 miles along the coast from the river mouth, where they were fed once daily to satiation with a wet diet consisting of beef liver, 4500 g; beef heart, 4500 g; canned salmon, 4500 g; salt, 110 g; and pablum, 40 g, and where they may also have picked up additional feed from the water that flowed through the pens as a result of tidal action. These captive fish were sampled at intervals until July 20. The range in salinity of Deep Bay water was 24.2–28.8‰ and the range in temperature was 12.0–18.4 C during the experimental period.

Sockeye were captured during the course of their movement from the Fulton River and Fulton River artificial spawning channel as fry in June 1966 and were recovered as unmarked underyearlings from Babine Lake by purse seining during the summer and early fall of 1966 as described by McDonald (1969).

Each sample of fish was anaesthetized with 2-phenoxyethanol; fork lengths and live weights were recorded and subsamples taken for each chemical analysis. Fish for analysis of total lipid were placed individually in small plastic bags, frozen on dry ice, and stored at −20 C for a few days until analyzed. Fish for moisture determination were placed in dried and tared aluminum dishes, and fish for total nitrogen analysis in acid-cleaned, large screw-top test tubes, prior to freezing. Each chemical analysis was performed on an individual fish: total nitrogen by the Kjeldahl method (Association of Official Agricultural Chemists, 1960); total solids by drying to constant weight at 105 C; total moisture by the difference between live and dry weights; and total lipid by the method of Folch et al. (1957).

The weight–length relation ($W = aL^b$),[1] lipid–weight relation ($F = cW^d$),[1] and the moisture–, dry weight–, and nitrogen–total weight relations ($Y = e + fW$), together with the type 2 errors of the parameters were determined by the method of least-square fit. Moisture, solids, lipids, and nitrogen in fish of the mean weight at each sampling time were determined from the least-square fit lines of these components on weight. The resultant fitted values of these components and the mean values for length and weight were plotted on a logarithmic basis against time ($\bar{Y} = gh^t$).

During periods of exponential growth the above plots against time formed relatively straight lines. Rates of growth in length and in weight, and the rates of increase in moisture, solids, lipid, and nitrogen were determined from the values of the parameter, h, of the calculated least-square fit lines through these plots. Similarly the fitted weights of moisture, solids, lipid,

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[1]W = fish weight (g); L = fork length (cm); $\bar{L}$ = mean fork length (cm); F = weight of lipid (mg); Y = weights of moisture, solids, or nitrogen (mg); $\bar{Y}$ = mean fork length (cm), mean weight (g), or fitted weights of moisture, solids, lipids, or nitrogen (mg); t = time (days); the lower case letters a–j are constants.

and nitrogen at each sampling time were plotted against mean length on a log–log basis and the value of the power, j, of the least-square fit line calculated ($\overline{Y} = i\,\overline{L}^{j}$).

RESULTS AND DISCUSSION

Growth in Length and in Weight and Weight–Length Relations

There were no apparent differences between the lengths or weights of channel and river chum salmon fry migrating to sea from May 2 to May 18 or between the lengths or weights of channel and river sockeye migrating to the lake from June 5 to June 14 (Fig. 1). After transfer to sea water on

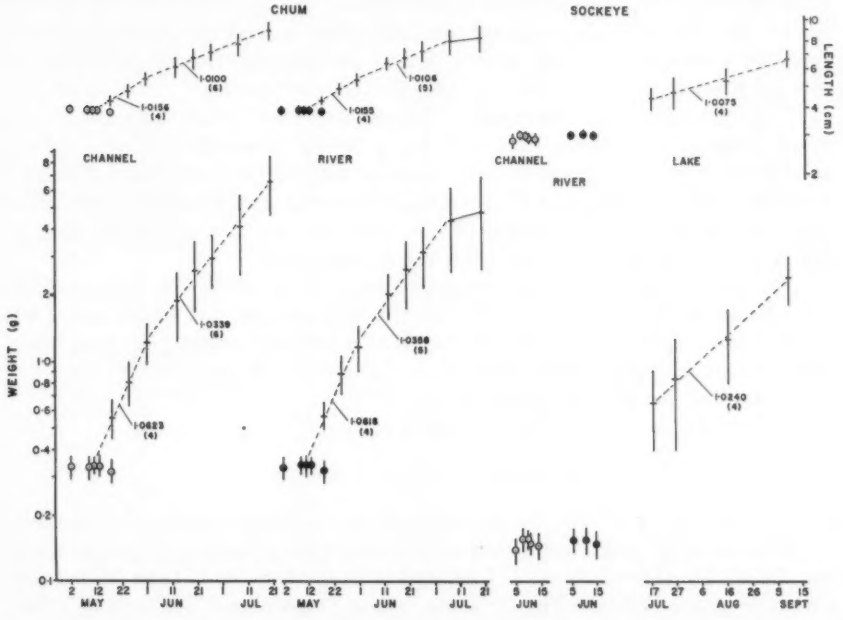

FIG. 1. Mean fork length and mean weight of migrating chum and sockeye fry and growth in mean fork length and in mean weight of postmigrant juvenile chum and sockeye salmon. Open circles represent migrating channel fish and solid circles migrating river fish. Crosses represent chum salmon migrants of May 11 confined to pens in sea water and sockeye salmon captured at intervals in Babine Lake. Broken lines represent the least-square fit lines of length or weight on time. The slopes of these lines, together with the number of points used in their calculation, are shown on the graphs. Vertical bars represent two standard deviations of each sample.

May 11 both channel and river chums grew exponentially with length increasing 1.6% per day and weight increasing 6.2% per day until June 1 after which growth in length and in weight remained exponential, but at decreased rates of 1.0% and 3.5% per day respectively. Most of the larger river chums escaped from the live pen after the sampling of July 8 and the length–weight data from the sample of July 20 was not included in the calculations for growth rates of these fish.

Le Brasseur and Parker (1964) obtained similar rates of growth and a similar change in the rates for young pink salmon after migration to sea as fry. These investigators found that there was an initial period (40 days) of fast growth in enclosed coastal waters during which length increased 1.88% per day and weight increased 6.24% per day. The termination of this period of rapid growth coincided with the exodus of the fish from the estuary when the fish were 6–8 cm long. The secondary growth period was characterized by growth rates of 1.09% and 3.58% per day in length and in weight respectively during the first 30 days the fish were in the open ocean. Le Brasseur (1969) determined the growth rate in weight of captive juvenile chum salmon fed to excess with a diet of mixed zooplankton over a 6-week period from May 31 to July 12. This investigator did not observe two growth stanzas and reported a rate of 5.4% per day, which is greater than the rate of 3.5% per day for the period from June 1 to July 20 reported herein.

The growth rates for captive channel and river chum salmon fry are much higher than those reported for yearling postmigrant coho salmon smolts maintained in sea water under identical conditions where length increased 0.4% per day and weight 1.6% per day (Vanstone and Markert, 1968). The different growth rates between these two species are probably due to differences in the age and size of the fish at migration.

Growth of sockeye underyearlings growing naturally in Babine Lake was also exponential with the rates being 0.75% per day for length and 2.4% per day for weight. McDonald (1969), using a different method of calculation, has reported a closely similar daily rate of growth in length (0.739%) per day for unmarked sockeye in Babine Lake.

The slope, b, of the weight–length formula:

$$\log \text{weight} = \log a + b \log \text{length}$$

was approximately 2.7 for the majority of the individual samples of channel- and river-hatched migrating chum salmon and was 2.62 and 2.75 respectively for pooled samples (Table 1). The pooled data from each type of fish are presented graphically in Fig. 2. From visual examination of the fish a few large migrants were present in the samples of May 13 and 18. The data of these individuals were not included in the values used to calculate mean lengths, mean weights, the least-square fit line of weight on length or chemical composition. However, the weight–length data of these seven fish, which are shown as individual points on Fig. 2, indicate that these fish were at a developmental stage between migrating fry and postmigrants. The slopes for samples of migrating channel sockeye ranged from 1.5 to 2.5 with a value for all channel migrants of 1.8, whereas the slopes for samples of migrating river sockeye were much higher with a range of 2.6–3.0 and a pooled value of 2.9 (Table 1, Fig. 2). These values for sockeye fry are comparable with the values ranging from 1.1 to 3.2 reported for seaward migrating pink salmon fry (Skud, 1955). Sockeye and pink fry are very similar in size and shape at this stage of their life cycle and similar values for the parameter b were not unexpected.

TABLE 1. Parameters and type 2 errors of the least-square fit relations of fish weight on fork length of various groups of chum and sockeye salmon (log weight, mg = log a + b log length, cm).

Date	Sample size	Least-square fit of weight on length ($W = aL^b$)			
		a (mg)	Error	b	Error
Channel chums, migrants					
May 2	80	6.8	2.2	2.87	0.24
9	80	8.8	2.7	2.69	0.23
11	80	13.2	3.5	2.40	0.20
13	77	9.1	1.9	2.68	0.16
18	77	8.3	2.6	2.74	0.23
Pooled May 2–18	394	9.7	1.2	2.62	0.09
Channel chums, migrants of May 11 held in seawater live boxes					
May 18	80	4.2	1.1	3.39	0.18
25	79	4.7	0.8	3.32	0.11
June 1	80	6.3	1.8	3.14	0.16
13	80	3.5	0.6	3.45	0.09
20	78	4.5	0.9	3.33	0.10
27	78	6.6	1.2	3.11	0.09
July 8	75	3.5	0.6	3.44	0.08
20	79	7.2	1.1	3.12	0.07
Pooled May 18–July 20	629	4.5	0.2	3.32	0.02
River chums, migrants					
May 2	79	6.7	1.9	2.90	0.21
9	80	13.0	3.2	2.40	0.19
11	80	8.9	2.2	2.69	0.18
13	79	7.0	1.9	2.87	0.20
18	80	6.9	2.0	2.87	0.22
Pooled May 2–18	398	8.2	1.0	2.75	0.09
River chums, migrants of May 11 held in seawater live boxes					
May 18	77	3.7	1.0	3.48	0.19
25	78	6.0	0.9	3.16	0.10
June 1	80	2.5	0.5	3.69	0.12
13	80	4.3	0.8	3.34	0.10
20	80	4.4	0.9	3.35	0.10
27	80	7.7	1.2	3.04	0.08
July 8	76	2.7	0.4	3.55	0.07
20	50	3.4	0.4	3.42	0.06
Pooled May 18–July 20	601	5.3	0.2	3.24	0.02
Channel sockeye, migrants					
June 5	80	30.8	4.9	1.46	0.15
8	80	10.2	2.6	2.50	0.23
10	80	27.3	6.6	1.62	0.22
11	80	29.6	2.6	1.53	0.18
14	80	19.7	3.1	1.91	0.15
Pooled June 5–14	400	23.0	1.9	1.76	0.08
River sockeye, migrants					
June 6	80	5.5	1.6	3.04	0.26
11	80	5.8	1.6	2.98	0.25
15	80	8.6	2.3	2.62	0.24
Pooled June 6–15	240	6.7	1.0	2.86	0.14
Lake sockeye					
July 18	75	4.7	0.6	3.29	0.08
27	75	5.5	0.6	3.20	0.06
Aug. 17	75	8.5	0.8	2.98	0.05
Sept. 10	75	5.3	0.7	3.22	0.07
Pooled July 18–Sept. 10	300	5.7	0.2	3.19	0.02

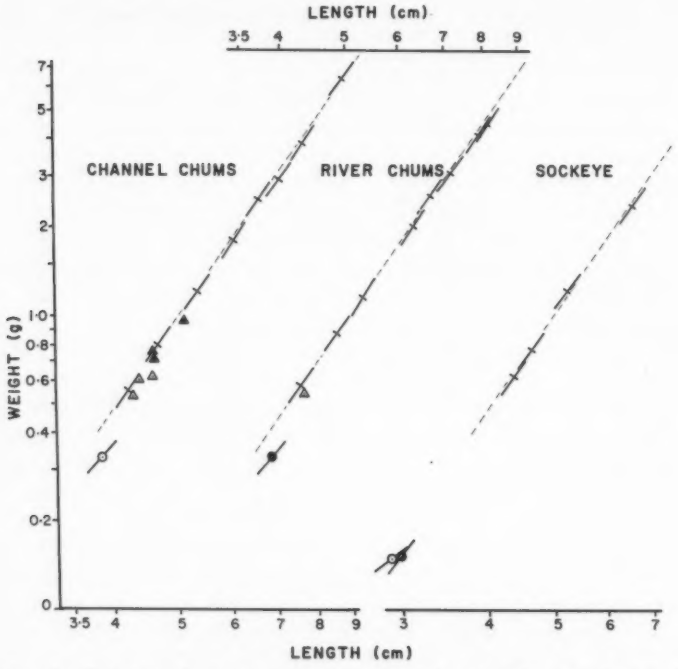

FIG. 2. Relations of mean weight on mean fork length of juvenile chum and sockeye salmon with a section of least-square fit line of weight on length for each sample. Circles and crosses have the same significance as those used in Fig. 1. Triangles represent individual migrating chum fry that were not included in the large samples used to determine the least-square fit relations: the four open triangles represent individual migrants of May 13 and the three solid triangles represent individuals of May 18. The broken line on each graph is included for reference purposes and its equation is: log weight, mg = log 5.2 + 3.25 log length, cm.

During captivity in seawater holding pens the chum salmon became heavier per unit length than migrants and the plots of mean weight against length of both groups of chums as well as those of sockeye in the lake clustered around the reference lines in Fig. 2, which have a value for b of 3.25. The actual value of the parameter, b, of individual samples of postmigrant chums and sockeye ranged from 3.0 to 3.7 with pooled values of 3.32, 3.24, and 3.19 for channel chums, river chums, and lake sockeye respectively (Table 1). These are comparable with the values of 3.25 reported by Le Brasseur and Parker (1964) for postmigrant pink salmon and 3.24 reported by Vanstone and Markert (1968) for postmigrant coho salmon.

CHEMICAL COMPOSITION

Total lipid was logarithmically related to fish weight in all samples examined (Table 2, Fig. 3) and, with the exception of migrating sockeye, large fish

TABLE 2. Parameters and type 2 errors of the least-square fit relations of total lipid on fish weight of various groups of chum and sockeye salmon (log lipid, mg = log c + d log weight, g).

Date	Sample size	Least-square fit of lipid on weight ($F = cW^d$)			
		c (mg)	Error	d	Error
Channel chums, migrants					
May 2	25	44.8	14.8	1.07	0.30
9	23	84.9	41.4	1.62	0.46
11	24	56.1	23.1	1.16	0.39
13	23	47.9	12.3	1.02	0.24
18	23	47.8	9.1	1.02	0.18
Pooled May 2–18	118	54.2	8.0	1.17	0.14
Channel chums, migrants of May 11 held in seawater live boxes					
May 18	25	46.2	2.2	1.39	0.09
25	23	46.0	1.7	1.45	0.16
June 1	24	42.9	1.5	1.20	0.11
13	25	34.3	2.7	1.39	0.09
20	24	29.9	4.1	1.45	0.11
27	25	33.3	4.3	1.37	0.11
July 8	23	32.2	4.5	1.26	0.08
20	24	23.5	6.5	1.42	0.13
Pooled May 18–July 20	193	39.3	1.4	1.17	0.02
River chums, migrants					
May 2	23	54.7	14.4	1.25	0.24
9	23	49.8	18.6	1.18	0.35
11	25	34.2	10.8	0.81	0.29
13	24	136.3	47.1	2.03	0.32
18	25	50.8	14.6	1.06	0.27
Pooled May 2–18	120	58.4	8.7	1.27	0.14
River chums, migrants of May 11 held in seawater live boxes					
May 18	24	40.2	4.4	1.23	0.19
25	24	43.8	0.7	1.30	0.08
June 1	24	47.3	3.6	0.88	0.26
13	25	34.8	5.1	1.41	0.17
20	25	39.8	4.8	1.22	0.10
27	24	34.3	4.2	1.33	0.09
July 8	23	17.2	5.1	1.71	0.16
20	15	19.5	5.7	1.55	0.15
Pooled May 18–July 20	184	35.7	1.8	1.28	0.03
Channel sockeye, migrants					
June 5	25	80.1	50.5	0.98	0.32
8	25	42.0	10.5	0.69	0.13
10	25	25.5	13.7	0.48	0.29
11	25	23.6	12.6	0.36	0.28
14	24	10.7	6.5	0.01	0.32
Pooled June 5–14	124	20.8	4.6	0.33	0.12
River sockeye, migrants					
June 6	25	23.5	14.0	0.46	0.32
11	25	182.5	68.2	1.55	0.21
15	23	38.2	12.4	0.64	0.17
Pooled June 6–15	73	42.7	11.6	0.75	0.15
Lake sockeye					
July 18	23	29.2	0.9	1.32	0.08
27	23	28.3	1.2	1.65	0.10
Aug. 17	24	28.1	2.3	1.47	0.15
Sept. 10	25	31.4	4.2	1.56	0.13
Pooled July 18–Sept. 10	95	27.9	1.3	1.66	0.05

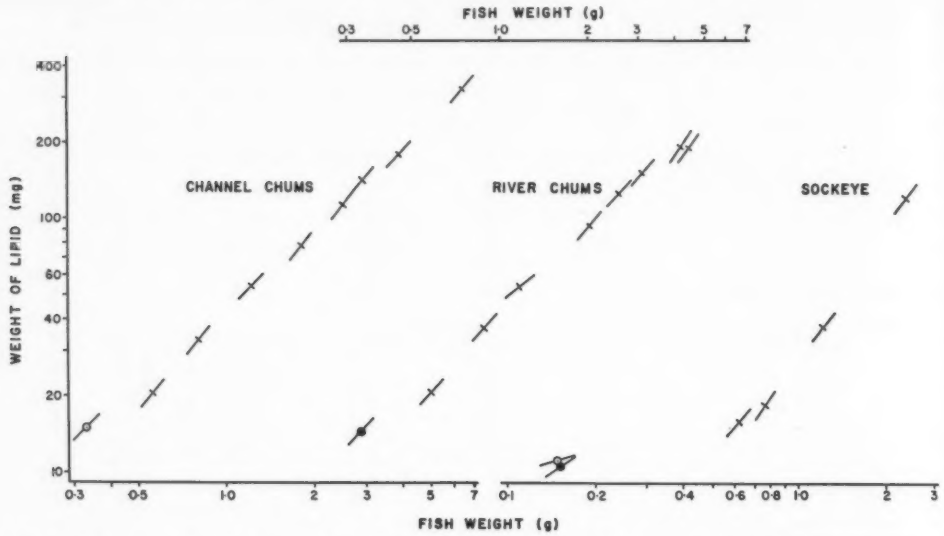

FIG. 3. Relations of mean weight of total lipid on mean fish weight of juvenile chum and sockeye salmon with a section of least-square fit line of lipid weight on fish weight for each sample. Circles and crosses have the same significance as those used in Fig. 1.

had a higher percentage lipid than small fish (power $d > 1$). Postmigrant fish of both species contained less lipid per unit weight than migrating fry. From the results of Parker and Vanstone (1966) on postmigrant pink salmon which, like chum salmon, migrate to sea shortly after emergence from the gravel, a decrease in lipid was expected in the chums approximately 6 weeks after migration. The absence of this phenomenon in the chum salmon and in postmigrant 1-year-old coho salmon held in seawater pens under identical conditions (Vanstone and Markert, 1968) cannot be explained.

Total moisture, dry weight, and nitrogen were linearly related to weight with inflections in each square fit line. These inflections occurred in chums at a fish weight of 2 g for all three components and in sockeye at a fish weight of 1.5 g for moisture and dry material and at 0.9 g for nitrogen. Similar size-dependent changes in the linear relations of these components on weight were reported previously for coho yearlings (Vanstone and Markert, 1968).

There were no apparent differences in the chemical composition of channel- or river-hatched chum salmon either migrating or held in seawater live boxes (Tables 2 and 3, Fig. 3 and 4). Although there was a suggestion that migrating river-hatched sockeye may contain more total nitrogen and less total lipid per unit weight or length than channel-hatched migrants, the difference, as well as those in the weight–length relations may be due to the 11-day difference in the timing of the peaks of the two fry migrations with channel fry migrating later than river fry (McDonald, 1969).

TABLE 3. Parameters and type 2 errors of the least-square fit relations of total moisture, dry weight, and nitrogen on live weights of various groups of chum and sockeye salmon (Y, mg = $e + f$ weight, g).

	Fish wt (g)	Sample size	e (mg)	Error	f	Error
Channel chums, migrants						
Moisture	–	123	7.1	2.3	786.5	6.8
Solids	–	123	−7.1	2.3	213.5	6.8
Nitrogen	–	103	0.2	0.5	21.9	1.6
River chums, migrants						
Moisture	–	124	6.9	2.8	789.9	8.4
Solids	–	124	−6.9	2.8	210.1	8.4
Nitrogen	–	103	−1.2	0.5	25.3	1.5
Channel sockeye, migrants						
Moisture	–	125	−13.3	2.1	881.1	14.5
Solids	–	125	13.3	2.1	118.9	14.5
Nitrogen	–	100	−1.9	0.4	34.9	2.6
River sockeye, migrants						
Moisture	–	75	−7.0	2.1	841.5	13.6
Solids	–	75	7.0	2.1	158.5	13.6
Nitrogen	–	60	−1.4	0.5	32.2	3.2
Channel chums, migrants of May 11 held in seawater pens						
Moisture	<2	100	15.4	2.8	793.2	2.6
	>2	96	74.7	9.5	760.7	2.1
Solids	<2	100	−15.4	2.8	206.8	2.6
	>2	96	−74.7	9.5	239.3	2.1
Nitrogen	<2	79	−3.2	0.3	26.8	0.3
	>2	75	−6.3	1.0	28.3	0.2
River chums, migrants of May 11 held in seawater pens						
Moisture	<2	101	12.6	3.1	795.8	2.7
	>2	87	109.3	8.2	748.4	1.9
Solids	<2	101	−12.6	3.1	204.2	2.7
	>2	87	−109.3	8.2	251.6	1.9
Nitrogen	<2	82	−3.0	0.5	26.3	0.5
	>2	71	−4.1	2.5	27.1	0.6
Lake sockeye						
Moisture	<1.5	92	23.5	2.3	790.0	2.9
	>1.5	33	127.4	16.3	726.3	6.9
Solids	<1.5	92	−23.5	2.3	210.0	2.9
	>1.5	33	−127.4	16.3	273.7	6.9
Nitrogen	<0.9	50	−0.6	0.4	22.0	0.7
	>0.9	45	−1.4	0.8	24.8	0.4

In both species of fish, growth in weight was accompanied by increasing amounts of the four components, but the rates of increase in moisture were less than the rates for total weight, solids, lipid, or nitrogen (Fig. 5). During the first period of exponential growth in sea water, whereas weight was increasing 6.2% per day in both channel and river chums, moisture increased 6.15% per day, dry matter 6.3% per day, lipid 6.5% per day, and nitrogen

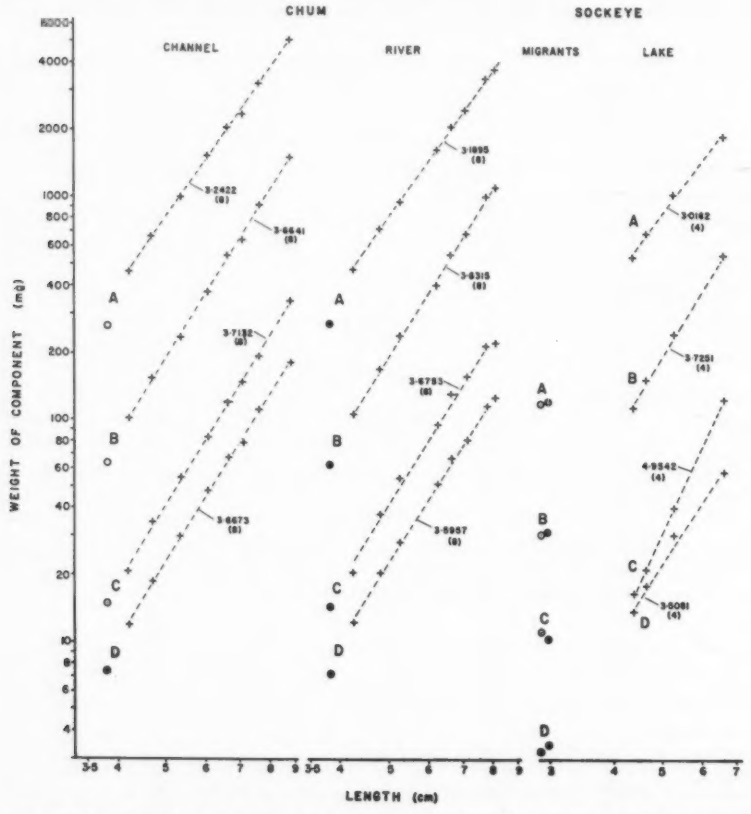

FIG. 4. Fitted weights of total moisture A, solids B, lipid C, and nitrogen D, in juvenile chum and sockeye salmon of the mean weight at each sampling time plotted against mean length of each sample. Symbols have the same significance as those used in Fig. 1. Broken lines represent the least-square fit lines of weight of each component on length. The slopes of these lines, together with the number of points used in their calculations, , are shown on the graphs.

6.7% per day. Similarly during the slower second period of exponential growth when weight was increasing 3.5% per day moisture increased 3.35% per day whereas dry matter, lipid, and nitrogen increased 3.9, 3.75, and 3.7% per day respectively. In a like manner whereas moisture in Babine Lake sockeye was increasing only 2.3% per day, total weight, solids, lipids, and nitrogen were increasing at the greater rates of 2.4, 2.8, 3.8, and 2.7% per day respectively. These results are similar to those previously reported for coho salmon (Vanstone and Markert, 1968) and are confirmed in Fig. 4 and Table 1 where increases in moisture per unit increases in length are less than the increases in total weight, dry weight, lipid, or nitrogen.

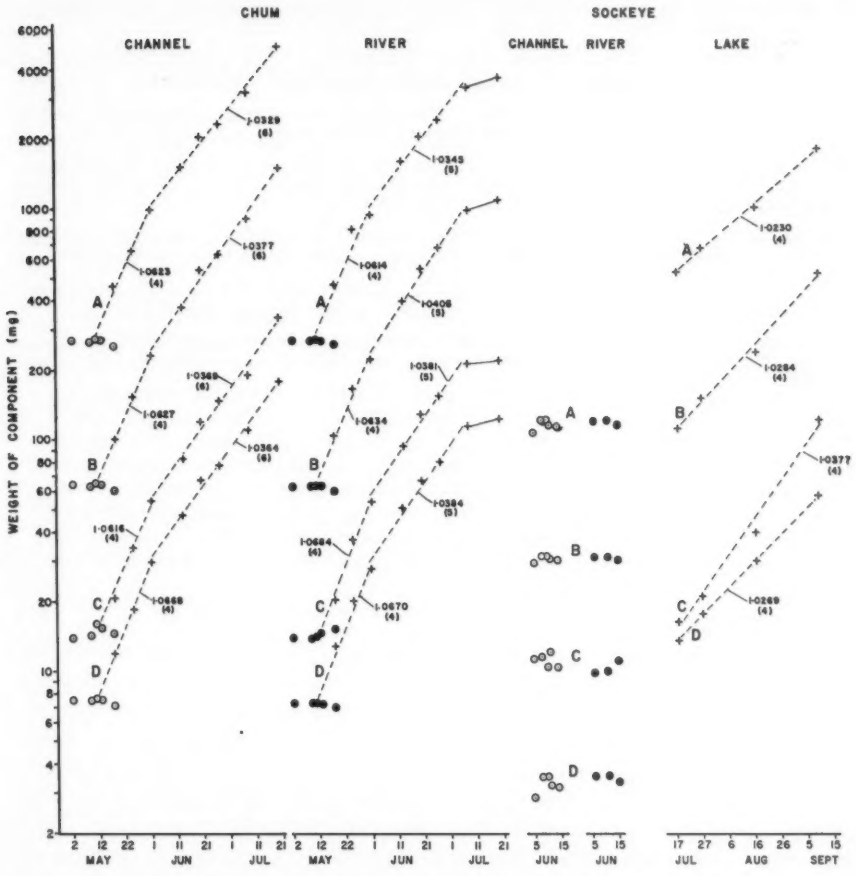

FIG. 5. Fitted weights of total moisture A, solids B, lipid C, and nitrogen D, in juvenile chum and sockeye salmon of the mean weight at each sampling time plotted against sampling date. Symbols have the same significance as those used in Fig. 1. Broken lines represent the least-square fit lines of weight of each component on time. The slopes of these lines, together with the number of points used in their calculations, are shown on the graphs.

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Effects of Metal Poisoning on Five Liver Enzymes in the Killifish (*Fundulus heteroclitus*)

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JACKIM, E., J. M. HAMLIN, AND S. SONIS. 1970. Effects of metal poisoning on five liver enzymes in the killifish (*Fundulus heteroclitus*). J. Fish. Res. Bd. Canada 27: 383–390.

Activities of five liver enzymes (acid and alkaline phosphatase, catalase, xanthine oxidase, and ribonuclease) from *Fundulus heteroclitus* surviving exposure to 96-hr TL_m concentrations of salts of six metals (lead, copper, mercury, beryllium, cadmium, and silver) differed markedly from those of unexposed fish. Changes in enzyme activity produced by the exposures were not necessarily the same in magnitude or direction as those observed when the salts were introduced directly into the enzyme preparations. It is proposed that changes in liver enzyme activity may be useful as a kind of biochemical autopsy tool for diagnosing sublethal metal poisoning in fish.

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ALTHOUGH THE STUDY OF CHANGES in enzyme activity can be an important aspect of mammalian toxicology, little has been done in this respect on fish. At this time the only practical enzyme test used in fish toxicology is the measurement of acetylcholinesterase inhibition by organophosphorus insecticides (Holland et al., 1962). Other past attempts to relate toxicity to changes in enzyme activity in fish have not been very successful. Nevertheless Leduc (MS, 1966), and Bell (1968) demonstrated changes in several fish enzymes as a result of exposure to cyanide or injection with carbon tetrachloride and bromobenzene.

This paper reports changes in activities of five liver enzymes after exposing fish or fish liver homogenates to salts of various toxic metals. Killifish were used exclusively in this study and enzyme activities were determined in fish surviving the metal exposure. Liver alkaline phosphatase, xanthine oxidase, and catalase were selected on the assumption that because they are metal-requiring enzymes, traces of added cations might displace or affect the metal containing moiety and modify enzyme activity. Ribonuclease was chosen because of its sensitivity to certain metals, and acid phosphatase was studied because of its involvement in mineral metabolism. Other reasons for selecting the particular enzymes were the relative ease of assay, stability, and availability of information for similar enzymes in other vertebrates. The prime object of the study was to determine the feasibility of a biochemical autopsy technique for diagnosing sublethal metal poisoning of fish. A peripheral objective was

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to gain insight into the mechanisms of metal poisoning. With this in mind, experiments were conducted in which metal salts were added directly to enzyme reaction mixtures as well as to aquaria containing fish. Thus, a comparison could be made between direct (in vitro) effects of metals on enzymes and the indirect (in vivo) effects as measured in liver preparations from animals exposed to metal salts.

MATERIALS AND METHODS

The test species was *Fundulus heteroclitus*. The fish were caught in local estuaries and held in fiber-glass tanks at 20–22 C with daily feeding of brine shrimp for a minimum of 3 days. They were collected between March and September from waters ranging between 5 and 24 C. Ten fish of mixed sex and similar size (3.5–5 cm) were placed in 20-liter jars containing sea water filtered through sand and gravel. Preliminary experiments indicated the effect of sex to be insignificant on the enzymes tested.

After 3 hr, a solution of a metal salt was added to each jar in an amount approximating the 96-hr TL_m concentration as follows:

Metal	Be^{++}	Cd^{++}	Cu^{++}	Pb^{++}	Hg^{++}	Ag^{+}
Concn (*mg/liter*)	31.0	27.0	3.2	188.0	0.23	0.04

Analysis of the bioassay water at the end of the test, by atomic absorption spectroscopy, revealed considerable decreases in concentration of the toxic elements, excepting cadmium, as follows:

Metal	Be^{++}	Cd^{++}	Cu^{++}	Pb^{++}	Hg^{++}	Ag^{+}
Final concn (*mg/liter*)	26	27	0.5	39.4	0.17	0.03
Loss (%)	16.1	0	84.4	79.1	26.1	25.0

Differences between the initial and final concentrations of elements added to the bioassay water in part resulted from uptake by the fish either internally or on its surfaces. Most of the decline, however, probably resulted from precipitation in sea water. Such factors as complexing with organic materials and adsorption on glass could also account for loss of the metal.

Experimental controls were run simultaneously under identical conditions except that no toxic cations were added to the water. Feeding was discontinued during the 96-hr test period. All test units were continuously aerated, glass-wool filtered, and kept at room temperature (20–25 C.) Dead fish were discarded twice daily. Surviving fish were removed from the jars at 96 hr or as indicated and quickly decapitated prior to dissection. The assay livers were immediately removed, and placed in precooled and tared beakers for weighing.

The homogenates used for acid and alkaline phosphatase, catalase, and xanthine oxidase assays were made by pooling the livers of five fish from each group. Weighed tissue was diluted 1:25 w/v with ice-cold distilled water followed by homogenization at 0 C by 15 passes through a Potter-Elvehjem type power-driven tissue grinder. This homogenate was then subjected to sonic cell disruption for 30 sec at 0 C using a Heat Systems Sonifier at 50–100 w. The homogenate was centrifuged for 1 min at 2000 rpm in a clinical centrifuge to remove any remains of connective tissue. Homogenates used for liver RNAase assays were prepared similarly except that 0.25 M sucrose was used for dilution instead of distilled water.

In all enzyme assays the buffer *p*H was selected to produce maximum or near maximum enzyme activity. The buffers used were the same as those from which the procedure was adapted. No further attempt was made to find buffers promoting optimal activity. Preliminary experiments showed that reaction rate gradually increased with temperature up to a critical point, at which the activity rapidly ceased. Consequently, the temperature at which the enzyme reactions were

conducted was a compromise between that for maximum activity and stability. In all instances it was determined that minor changes in temperature encountered during the course of the experiment would have no significant effect on the reaction rate. The time intervals used in the enzyme assays were within the linear portion of the reaction curve, ensuring that the rate was limited by enzyme concentration and not by availability of substrate or product formation. A Beckman DB-G spectrophotometer was used for all optical density measurements.

In the assay for acid and alkaline phosphatases, flavone-3-phosphate was used as substrate (Land and Jackim, 1966). Alkaline phosphatase activity was measured colorimetrically at 26 C for 12 min, and acid phosphatase was measured over a 25-min period. The reference cuvette contained 2.9 ml buffer and 0.1 ml homogenate. With in vitro experiments the buffer contained the toxic metal salts. The final values for enzyme activity were expressed as the changes in optical density per milligram of protein over the specified incubation period.

Xanthine oxidase was assayed by the reduction of 2,6-dichlorophenol indophenol (Mahler et al., 1954). The sample cuvette contained 2.9 ml of 0.06 mM indophenol solution that included 0.1 mM xanthine (xanthine solution made fresh daily), and 0.1 ml tissue homogenate. The reference cuvette contained 2.9 ml of 0.06 mM indophenol without xanthine and 0.1 ml homogenate. Indophenol solution was prepared by adding 2,6-dichlorophenol indophenol to 0.01 M phosphate buffer, *p*H 7.5. Change in absorbance was measured at 600 nm and 31 C. The results were reported as changes in optical density per milligram protein for 2.5 min.

In vitro effects of metals on RNAase activity were measured by the method of Ambellan and Hollander (1966). To 0.2 ml of 0.2 M acetate buffer *p*H 7.5, containing 7.5 mM EDTA, and the toxic metal salt were added 0.2 ml of 1% ribonucleic acid solution and 0.2 ml of homogenate. After 5 min incubation at 26 C the reaction was stopped with 6 ml of lanthanum nitrate precipating reagent and the reaction mixture was centrifuged at 10,000 rpm for 15 min. The reference control contained all reagents but was stopped at zero time. Optical density readings were made at 260 nm and the final activity was calculated as changes in optical density per 5 min per milligram protein.

RNAase assay for the in vivo study was identical except that the enzyme reaction was carried out at *p*H 7.8 in 0.05 M barbiturate buffer.

The assay for catalase was that described by Bergmeyer (1965). Activity was determined as the change in optical density per milligram of protein over a 24-sec time interval at 26 C. Changes in optical density were recorded at 240 nm. Three ml of phosphate buffer and 0.01 ml of the tissue homogenate were added to the reference cuvette. The sample cuvette had 3 ml buffer containing 1.25×10^{-2} M hydrogen peroxide. Toxic metal salts were added to the buffer for in vitro assays.

Differences between the means of control and experimental results were analyzed for statistical significance by the student's *t*-test.

RESULTS

The in vitro alkaline phosphatase study showed that beryllium and cadmium were the strongest inhibitors of this enzyme, with copper, lead, mercury, and silver producing progressively less inhibition (Table 1). In vivo beryllium and silver inhibited the enzyme in *F. heteroclitus* exposed to 96-hr TL_m concentrations; mercury and lead increased the activity whereas copper and cadmium had no significant influence (Table 2).

Lead, copper, silver, mercury, and cadmium added directly to the enzyme preparation (in vitro) inhibited acid phosphatase activity in decreasing order (Table 1). Except for silver, in vivo, exposure to all heavy metal salts inhibited liver acid phosphatase; silver failed to produce a significant change in activity (Table 2).

TABLE 1. Percentage enzyme inhibition resulting from in vitro addition of metal salts.

Molar concn of element	% inhibition					
Alkaline phosphatase						
	Be	Cd	Cu	Pb	Hg	Ag
10^{-7}	10	1	–	–	–	–
10^{-6}	36	27	0	–	1	–
10^{-5}	62	72	12	50	10	10
10^{-4}	70	80	36	64	68	53
Acid phosphatase						
	Pb	Cu	Ag	Hg	Cd	
10^{-7}	0.1	–	–	–	–	
10^{-6}	15	1	–	–	–	
10^{-5}	30	30	15	2	–	
10^{-4}	50	60	50	40	–	
10^{-3}	75	70	76	70	–	
10^{-2}	100	95	89.5	95	11.5	
Xanthine oxidase						
	Cu	Hg	Ag	Pb	Cd	
10^{-9}	3.0	–	–	–	–	
10^{-8}	15.1	–	–	–	–	
10^{-7}	39.3	27.2	–	–	–	
10^{-6}	69.6	67.2	25	1.8	–	
10^{-5}	81.8	88.2	75	36.4	–	
10^{-4}	100.0	100.0	100	63.4	–	
Catalase						
	Hg	Ag	Pb	Cd	Cu	
10^{-6}	20	–	–	–	–	
10^{-5}	32	27.5	4	1.4	–	
10^{-4}	57.5	53.5	29	6.2	1	
10^{-3}	100.0	80.0	50	14.1	12	

The direct effects of metal salts on xanthine oxidase preparations varied from very strong inhibition with copper to marginal inhibition with cadmium (Table 1). Xanthine oxidase activity of exposed fish was inhibited by all metals except copper, which increased xanthine oxidase activity (Table 2).

RNAase activity was inhibited by all the metals in vitro, the percentage decreases in optical density in the presence of 10^{-4} M concentrations of the salts being: lead, 27.4; copper, 18.0; cadmium 12.6; silver, 4.6; mercury, 4.3. All in vivo exposures to the same metals inhibited RNAase activity by 72 hr following an initial surge of activity (Fig. 1).

Mercury and silver were the most potent inhibitors of catalase followed by lead, cadmium, and copper in that order (Table 1). Fish exposed to 96-hr

TABLE 2. Ranges and means of enzyme activity from 10 fish surviving 96-hr TL_m concentrations of the toxic metals. The values are of changes in optical density over the specific reaction period. All differences between the control and experimental means are significant ($P < 0.05$) except those marked with an asterisk (*).

	Range	Mean
Alkaline phosphatase		
Control	.275–.319	.293
Copper	.243–.361	.325*
Mercury	.283–.350	.317
Lead	.294–.363	.316
Cadmium	.268–.302	.282*
Silver	.238–.285	.259
Beryllium	.240–.268	.251
Acid phosphatase		
Control	.130–.147	.139
Lead	.106–.112	.108
Cadmium	.108–.117	.112
Copper	.112–.128	.120
Mercury	.124–.133	.129
Silver	.135–.152	.144*
Xanthine ozidase		
Control	.150–.171	.160
Lead	.095–.115	.103
Mercury	.101–.118	.109
Silver	.109–.124	.117
Cadmium	.138–.148	.143
Copper	.190–.220	.203
Catalase		
Control	.167–.185	.173
Silver	.108–.125	.117
Lead	.117–.126	.122
Copper	.116–.143	.129
Mercury	.133–.152	.140
Cadmium	.130–.154	.143

TL_m concentrations of the heavy metals exhibited a decrease in liver catalase activity (Table 2).

DISCUSSION

The results of this study suggest that liver enzyme activities can be used as indicators of sublethal exposure of fish to toxic metals. Among the more consistent changes observed were the decrease in catalase with exposure to all metals tested, the decrease in RNAase with all metals except mercury,

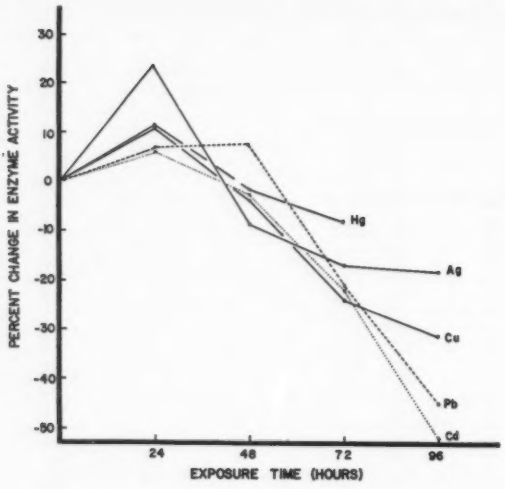

FIG. 1. Percentage changes in activity of fish liver RNAase exposed to salts of mercury, silver, copper, lead, and cadmium for various periods and assayed at pH 7.8. Each point represents the mean value for 10 fish.

and the decrease in acid phosphatase with all metals except silver. Decreases in xanthine oxidase activity with lead, mercury, and silver exposure and increase with copper were also important as was the decrease in alkaline phosphatase with beryllium. Inhibition of alkaline phosphatase by beryllium has been reported also in mammals: in isolated enzyme (Klemperer et al., 1949; Grier et al., 1949) and in rats (Cochran et al., 1950).

Because high concentrations of metals were required to inhibit catalase in vitro, no cation can be singled out as a specific inhibitor, even though mercury and silver are somewhat more active than other elements tested (Table 1). Catalase shows a striking difference between control and exposed fish for all metals tested. In this instance, the range of values of the experimental groups fell completely outside of the control range (Table 2). Because there was no overlap between the control and the experimental values, catalase activity could conceivably be used in diagnosis of fish suffering from metal poisoning. However, it should be established that other toxins or environmental factors do not induce a similar inhibition of this enzyme. As an example of such a possibility Kobayashi (1965) found that liver catalase activity increased when salmon smolts (*Oncorhynchus masou*) were transferred from fresh water to brackish water. This clearly demonstrates the complexity of enzymatic responses as they relate to changes of the external media.

Xanthine oxidase might be valuable in distinguishing copper toxicity from that of lead, mercury, silver, and cadmium because copper increases the activity in exposed fish whereas other metals inhibit it (Table 2). Differences of this kind could offer a possibility for distinguishing toxicity of one metal from another, provided that other products do not elicit a similar response. It is therefore obvious that screening with other groups of toxicants is necessary before this or other enzyme responses can be used for specific diagnosis.

Though identifying and measuring relative responses of enzymes to toxic metals was the object of this study, the physiological implications of responses to metals cannot be overlooked. As a cautionary note, however, it should be stated that the enzyme changes encountered were not of sufficient magnitude and consistency to be considered as immediate causes of toxicity. In addition, it must be remembered that all of the test fish appeared to be relatively healthy and not moribund after the exposures. It is conceivable that fish in a more deteriorated condition might show greater enzyme changes.

Toxin-induced changes in enzyme activity could represent the initial disorders ultimately resulting in death, or they can simply be symptoms of metabolic impairment(s). No attempt was made to differentiate symptomatic changes from causative changes in this study.

It is notable that copper, the strongest inhibitor of xanthine oxidase in vitro, enhances enzyme activity in vivo. Also, such differences in enzyme responses associated with in vivo and in vitro addition of metals suggests that different molecular processes are involved in the two types of exposure. Lack of correlation between in vivo and in vitro inhibition could be explained by cellular or tissue barriers that could exclude specific cations from active sites. In vivo activation could be attributed to enzyme induction by specific cations or indirectly through endocrine mechanisms such as those demonstrated in mammals (Knox et al., 1956). Other mechanisms could be postulated such as interactions with regulators or cofactors. The biphasic response of RNAase to metal toxicity (Fig. 1) could be envisioned as an initial induction or stimulation followed by direct inhibition as the toxic cation concentration increases in the tissues.

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NOTES

The Morid Fish *Halargyreus johnsonii* from the New York Bight

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HAEDRICH, R. L., AND M. H. HORN. 1970. The morid fish *Halargyreus johnsonii* from the New York Bight. J. Fish. Res. Bd. Canada 27: 391–393.

Three specimens of *Halargyreus johnsonii* Günther, 1862, 190, 345, and 381 mm standard length were trawled in 700 fath at 39°09′N, 72°11′W, a westerly range extension of some 1200 miles. The two larger specimens were just barely mature. The stomachs of all three were everted; the lower intestine of the largest specimen contained some grit. The largest specimen was host to a caligoid copepod. In addition to *Halargyreus*, the trawl captured 515 individuals in 22 species of deepwater fishes.

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HALARGYREUS JOHNSONII GÜNTHER, 1862, has its center of abundance in the North Atlantic from south and west of Iceland to the Faeroes Shelf and Ireland. Generally considered rare, it is sometimes taken in fair numbers with bottom trawls fished between 700 and 800 m deep (Krefft, 1966, 1967). The fish is not known from southern Greenland, but three specimens, heretofore the only western Atlantic records, have been reported from the Grand Bank (Templeman, 1968). In March 1969, three additional specimens were taken by RV *Chain* in the offings of Hudson Canyon at a depth of 700 fath (1280 m). These represent a range extension of some 1200 miles to the west. The species may thus occur sparingly in the western North Atlantic along the whole of the continental slope from the Grand Bank to the New York Bight.

The three specimens are 190, 345, and 381 mm in standard length (SL). They are deposited in the United States National Museum.

Circumstances of capture — RV *Chain* Cruise 88, trawl 7, 39°09.2′N, 72°11.5′W, March 6, 1969, 0930–1220 hr; time on bottom about 1 hr, 700 fath, 40′ Gulf of Mexico shrimp trawl.

Identification — Templeman (1968), in a comprehensive review of *Halargyreus*, concludes that all known representatives belong to one species, for which the oldest available name is *H. johnsonii* Günther, 1862. The three specimens here reported agree in every respect with both the generic diagnosis offered by Templeman (1968, p. 888) and with details in his radiographs, in

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which the spatulate first neural spine and the rudimentary spine at the anterior base of the first dorsal stand out particularly. There is a bony protuberance on the chin, but no barbel, and the vomer and palatines are toothless. All proportions and counts fall in or near Templeman's ranges for *H. johnsonii*. The scales in the lateral line number about 123 in the 190-mm specimen, about 122 in the 345-mm specimen, and about 120 in the 381-mm specimen.

Colour — When fresh, the specimens were a pale bluish-brown, with noticeably darker pigment around the eyes, lips, and gill openings. Sometime after preservation, the colour of the flesh itself became more apparent, and the specimens appear a light brown or even pale orange on the sides and back. The mouth, branchial cavity, and peritoneum are black.

Natural history — Both large specimens of *H. johnsonii* from Hudson Canyon appear to be females. The gonads, however, are not particularly well developed, and eggs cannot be detected in them. It is most probable that they are recently spawned out, although, to judge from the size of the gonad, the spawning cannot have been a very big one. Templeman (1968) suggests that specimens of *Halargyreus* considerably larger than the largest now known (432 mm SL) must exist. The state of the gonads of the 345- and 381-mm SL specimens here reported support his contention. The 190-mm specimen is immature.

The stomachs of all three specimens were everted, and hence nothing could be told concerning the food habits. In the lower part of the intestine of the largest fish there were small pieces of grit, suggesting some feeding on the bottom. Templeman (1968) believed that the projection of the lower jaw implied pelagic feeding.

The largest *H. johnsonii* was host to a large parasitic copepod, tentatively assigned to the order Caligoida. The site of attachment was on the left flank slightly behind the origin of the first dorsal fin and a short distance below the lateral line. Parasitic copepods were also found on two other species in the same trawl haul, *Antimora rostrata* and *Alepocephalus agassizi*. These copepods are under further study by Dr Sneed B. Collard at the University of West Florida.

With respect to the fish catch, the trawl haul that took the three *H. johnsonii* was fairly diverse. Included were: 3 *Centroscymnus coelolepis* (one measured) 735 mm total length (TL); 2 *Centroscyllium fabricii* (not measured); 4 *Raja bathyphila* (one measured) 450 mm TL; 1 *Harriotta raleighana* 619 mm TL; 1 *Hydrolagus affinis* 865 mm TL; 67 *Alepocephalus agassizi* 125–460 mm SL; 2 *Rouleina? sp.* 210 and 375 mm SL; 3 *Aldrovandia affinis* 375–470 mm SL; 6 *Aldrovandia phalacra* 265–ca. 390 mm SL; 3 *Polyacanthonotus rissoanus* 400–435 mm SL; 2 *Simenchelys parasiticus* 365 and 545 mm SL; 71 *Synaphobranchus pinnatus* 460–650 mm SL; 141 *Antimora rostrata* 201–502 mm SL; 16 *Urophycis chesteri* (not measured); 112 *Macrourus bairdii* 90–185 mm SL; 3 *Cottunculus thompsoni* 330–345 mm SL; 1 *Paraliparis sp.* 115 mm SL; 18 *Lycodes atlanticus* 217–362 mm SL; 1 *Lycodes vahlii?* 465 mm SL; 1 *Melano-*

stigma atlanticum 165 mm SL; 52 *Dicrolene intronigra* 184–354 mm SL; and 5 *Dibranchus atlanticus* (4 measured) 142–210 mm SL.

The so-called Shannon-Weiner information function (an index of diversity, $H = -\Sigma p \ln p$) was calculated for this assemblage. The value of H was 2.06, which is considerably lower than the H (>4) calculated for the assemblage of polychaetes and bivalves living in the bottom of the same area, the lower continental slope south of New England (Sanders, 1968).

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Record Size and Age of Atlantic Capelin, *Mallotus villosus*

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A female capelin caught in Trinity Bay, Nfld., in March 1967 represents a record size (252 mm total length) and age (10 years) for Atlantic capelin. It is suggested that this capelin was maturing for the first time.

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DURING a pleuronectid survey in early March 1967 the research vessel *A. T. Cameron* caught quantities of overwintering capelin in two bottom sets on the northern side of Trinity Bay, Nfld. Among the catches was a female capelin measuring 252 mm in total length and weighing 59 g in whole wet weight. The average diameter of the eggs in the ovaries ranged from 0.35 to 0.37 mm, which indicated that the fish was maturing and would spawn during the June–July spawning period. No residual eggs were present in the body cavity and

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the stomach was empty. The vertebral count of the specimen, excluding the basioccipital bone, was 66, the dorsal fin had 14 rays, and the left pelvic fin had 8 rays. Examination of the otoliths (Fig. 1) revealed that the capelin was 10 years old, i.e. of the 1957 year-class.

The previous maximum size and age of capelin in the Newfoundland area was a partially spent 5-year-old female capelin measuring 223 mm in 10% formalin (Templeman, 1948). Templeman estimated its fresh length to be around 230 mm. According to Prokhorov (1965) the largest capelin recorded in the Barents Sea was 210 mm in fork length (ca. 225 mm in total length) and the maximum age was 6 years. Vilhjaĺmsson (1968) recorded a maximum size of 195 mm total length and a maximum age of 5 years for the Icelandic capelin. In Greenland waters Hansen (1943) recorded a maximum size of 200 mm total length and a maximum age of 7 years. Thus, the above Trinity Bay specimen represents a new record size and age of Atlantic capelin.

The large size and old age of the record Trinity Bay capelin are inconsistent with the retardation of growth due to maturation and the high mortality often associated with repeated capelin spawning (Prokhorov, 1960). Also the absence of residual eggs suggests that it had not spawned during the previous year. It is therefore possible that the above specimen was maturing for the first time.

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(FIG. 1 *opposite*)

FIG. 1. Otoliths of 10-year-old capelin from Trinity Bay, Nfld., March 1967. ×20.

Reproduction and Growth of Smallmouth Bass, *Micropterus dolomieui*, in a Precambrian Lake

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Nest building by smallmouth bass in Tadenac Lake, Ontario, began in early June at 15–18 C and lasted less than 1 week. Nests were constructed at 0.3–2.4 m from the leaward shore. Egg incubation lasted 4 days at 15.2–18.2 C. Postlarval fish rose off nests at 8–11 days after hatching. Schools of young fish dispersed at 19–28 days. Both sexes matured at ages IV (18.7–25.0 cm) to VI (26.4–36.6 cm). Oldest fish (41.2 cm) were age IX. Temperature and shelter in the epilimnion determined summer distribution of fish of all ages. Annual littoral harvest (1963–66) averaged 4.0 k/ha, or 1.1 fish per angling hour.

Received March 3, 1969

BECAUSE OF WORLDWIDE INTEREST in extending the world distribution of the smallmouth bass (*Micropterus dolomieui*) to provide sports fisheries, documentation of the biology of a self-sustaining population of the species existing near the northern limit of its native range should prove useful for persons considering introductions into waters of temperate or subtemperate climates.

Observations on reproduction and growth of the species were made during 1965 and 1966 in Tadenac Lake, located within the Precambrian Shield of Ontario (45°04′N, 80°57′W). Because the lake is on a private, undeveloped tract of land not serviced by roads, it provided an excellent opportunity to observe smallmouth bass in a natural environment that has remained largely immune from ecological change associated with human activity.

Methods — The topography of Tadenac Lake was defined by means of a Bendix depth finder. Daily water temperatures were recorded with Taylor maximum–minimum thermometers at representative stations in the epilimnion. A Yellow Springs Thermistor thermometer was used for determining spot temperatures. Air temperatures were provided by the Meteorological Branch, Department of Transport of Canada.

Dissolved oxygen and free carbon dioxide concentrations were determined with a standardized Hack Oxygen Kit, model OX-2P, and a Hack Carbon Dioxide Kit, model CA-1P. A Hack Portable Engineers Laboratory, standardized by conventional methods (American Public Health Association, 1962), was used to determine concentrations of ammonia nitrogen, nitrate nitrogen, phosphate, and total alkalinity (calcium carbonate equivalents). Total dissolved solids were calculated using a Beckman RA-2A conductivity meter.

Nests were located and observations on spawning behaviour made from boat or shore positions. In 1966, 20 nests were selected for special study of the various phases of reproduction, particularly fry emergence and development.

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Growth data were obtained from fish taken with gill-, seine, dip-, and hoop nets, by spot poisoning with rotenone, or by angling between early spring and late autumn. Both total length (tip of snout to furthest tip of compressed caudal fin) and fork length (tip of snout to deepest caudal fin indentation) were recorded along with weight, sex, and maturity.

Age was determined by examining plastic impressions of scales taken from the left side of the fish, just posterior to the pectoral fin below the lateral line. Fish taken in early spring with extensive scale growth on the outer margin, but with no annulus, were considered to have one more annulus than was present. Regression equations for length–weight data on smallmouth bass were calculated according to the method of Beckman (1948).

Description of lake — Tadenac Lake has a surface area of 308.2 ha (761 acres) and a drainage basin of 829 ha (3.2 sq miles). Lake water level is maintained by a dam that separates the lake from Georgian Bay of Lake Huron and prevents fish movement into the lake. Approximately 40% of the lake is less than 9.0 m (29.5 ft) deep, corresponding approximately to the lower limit of the epilimnion. The maximum depth is 33.9 m (108 ft). Thermal stratification began about mid-May and ended in October. Surface water temperatures in 1965 and 1966 were positively correlated with ambient air temperature ($r = 0.74$). The epilimnion reached a maximum depth of 6.0–6.5 fath with surface temperatures (at 0.6 m) occasionally reaching 25.5 C.

Tadenac Lake is located within the continental climatic region characterized by cool summers and cold winters. Mean annual minimum and maximum air temperatures are –11 C and 32.2 C, respectively. The annual growing season (days with water temperature above 5.6 C) is 190 days. Annual precipitation is 86.4 to 91.4 cm (Department of Mines and Technical Surveys, 1957).

The shoreline is either wooded or faced with bare rock. The lake bottom within the littoral zone is approximately 31% sand or gravel; the remainder is composed of silted and weedy bays, or igneous rock, which forms barren shoals or drops off precipitously.

The epilimnion maintained dissolved oxygen saturation, carbon dioxide concentrations of less than 10 mg/liter, and a *p*H of 6.7–6.9 (Table 1). Maximum ammonia and nitrate nitrogen concentrations were 1.45 and 1.0 mg/liter, respectively. The highest recorded orthophosphate concentration was 0.24 mg/liter. Total alkalinity ranged from 17 to 30 mg/liter (calcium carbonate equivalent), and total dissolved solids from 38 to 50 mg/liter.

Observations and discussion — Nest building began on June 3 in 1965 and June 5 in 1966 when water temperatures reached 18 and 15 C, respectively. The activity lasted for less than 1 week (Fig. 1). Similar short nest-building periods and ambient water temperatures were reported by Hubbs and Bailey (1938), Surber (1942), and Latta (1963), although Rawson (1945) noted water temperatures as high as 20.6 C in Saskatchewan before nest building began.

Nests were constructed typically on sand or gravel at a water depth of 0.6–1.2 m and at a distance of 0.3–2.4 m from the leeward shore. Hubbs and Bailey (1938) noted a similar proximity of the nests to shore and Latta

TABLE 1. Concentrations (mg/liter) of various chemical constituents at various depths in Tadenac Lake, August 20, 1965, and March 9, 1966.

Constituent	Season	Surface (0.6 m)	Above thermocline (6.1 m)	Middepth (10.9 m)	Below thermocline (9.2 m)	Bottom (21.7 m)
NH_3	Summer	0	0	–	0.08	0.39
	Winter	1.45	–	0.96	–	0.70
NO_3	Summer	0.2	0.3	–	0.3	1.0
	Winter	0.62	–	0.78	–	0.96
PO_4	Summer	0.01	0.07	–	0.05	0.18
(ortho)	Winter	0.24	–	0.08	–	0.08
Total alkalinity	Summer	22	29	–	29	34
	Winter	17	–	24	–	30
Total dissolved	Summer	38.5	38.3	–	38.0	43.1
solids	Winter	50	–	38.0	–	45

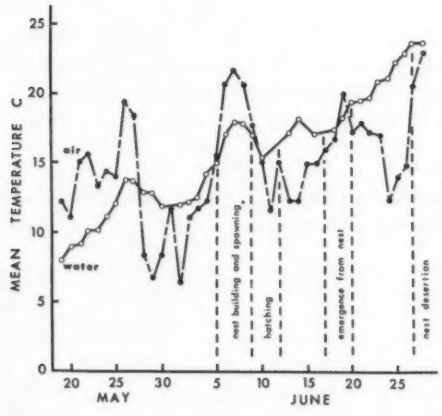

FIG. 1. Phases in the reproduction of smallmouth bass as related to water temperatures and time in Tadenac Lake, 1966.

(1963) noted the avoidance of prevailing winds in nest building. When adequate gravel was not available, the bottom of the concave bowl of each nest was usually covered with woody debris or broken clam shells, or both. Totals of 40 and 50 nests were seen in 1965 and 1966, respectively.

Egg incubation lasted 4 days at water temperatures ranging between 15.2 and 18.2 C (Fig. 1). Prolarvae, with a visible yolk sac, began to hatch on June 9 in 1966 and had all hatched by June 12. Tester (1930) and Rawson (1938) observed similar incubation periods in Lake Nipissing (16.7–20.0 C) and Lake Waskesiu (18.1–21.1 C), respectively. However, Beeman (1924) and Langlois (1932) reported incubation periods of up to 11 days at similar temperatures in New York and Ohio, respectively.

Of 20 nests examined in detail in 1966, 9 produced fry. Applying this ratio to the total number of nests seen in 1966 suggests that about 22 nests produced the 1966 year-class. There was no obvious explanation of why some nests failed and were deserted by the guarding male. Although fungus typically developed in deserted nests containing dead eggs, it also occurred in nests that ultimately produced young fish.

Postlarval bass, as defined by Lagler (1956), rose off the nests in 1966 at 8–11 days after hatching, between June 17 and 20, when water temperatures ranged between 17.2 and 19.5 C. Similar observations were made by Tester (1930) and Rawson (1938).

After rising from the nest, the young free-swimming bass remained in a dense mass close to the bottom and directly over the nest. However, within 2 days they began to scatter slightly but still remained more or less over the nest. Five days after rising, bass ranged beyond the perimeter of the nest to about 1 m and by 10 days to about 5 m. Gradually each aggregation of young became less concentrated and extended its range further from the nest while still accompanied by the protecting male. Between 19 and 28 days after hatching, both the guarding male and the young fish dispersed from the vicinity of the nest.

Young-of-the-year fish averaged 35.2 mm by the end of August, slightly larger than that recorded for nearby Lake Nipissing (Tester, 1930) and Georgian Bay (Doan, 1940). However, annual growth between ages I and IX was similar to that reported for the species from other waters within their native range (Table 2).

TABLE 2. Mean lengths (cm) at various ages of smallmouth bass in Tadenac Lake and other waters.

Age	Tadenac Lake (1965)		Tester (1930)		Doan (1940)	Webster (1954)	Latta (1963)
	TL[a]	SL[a]	Georgian Bay SL	L. Nipissing SL	Georgian Bay SL	Cayuga L., N.Y. TL	L. Michigan TL
I	9.05(4)	7.50	–	–	–	–	9.9
II	16.31(3)	13.4	–	18.0	–	16.3	16.0
III	18.9(17)	15.7	–	20.7	14.8	21.3	20.5
IV	23.0(23)	19.0	–	23.9	22.0	26.2	24.6
V	29.1(43)	24.0	21.7	25.7	23.2	34.7	29.2
VI	31.5(51)	26.0	24.6	29.6	25.7	34.8	33.5
VII	36.2(21)	29.7	25.2	30.7	27.9	37.3	37.1
VIII	24.9(4)	0.6	27.8	32.8	31.2	39.6	40.1
XI	41.2(2)	33.7	–	33.2	–	42.4	42.7

[a]TL = total length; SL = standard length.

The regression equations of weight on length (Beckman, 1948) for young-of-the-year ($\log W = -4.1770 + 2.7010 \log L$) and older bass ($\log W = -5.1220 + 3.2047 \log L$) compared favourably with those determined by Beckman (1948) and Latta (1963) for bass in Michigan waters.

Bass of both sexes began to mature at age IV (18.7–26.0 cm) with 33.3% of the males and 9.1% of the females mature. Of fish of age V, 88% of males and 82% of females were mature. All fish of age VI (26.4–36.6 cm) examined were mature. Hubbs and Bailey (1938) also found that both sexes matured between ages IV and VI in Michigan waters as did Stone et al. (1954) for fish from the Lake Ontario and St. Lawrence River region.

The distribution of smallmouth bass in Tadenac Lake, based on angling and netting data, was limited, as in the Finger Lakes (Webster, 1954), to the epilimnion during the period of summer stratification. Water temperatures in the epilimnion ranged from 7.2 to 27.2 C during 1965 and 1966. As the preferred temperature of the species is between 20.3 and 21.3 C (Bennett, 1965), only an estimated 10–15% of the bottom area of the epilimnion was utilized regularly by the species during the summer months. The fish tended to stay in the shelter of rock cliffs, overhanging shore-line brush and trees, and submerged boulders, stones, and logs; they occupied largely only the littoral areas of mainland, islands, and rocky shoals within the epilimnion.

Annual creel records indicate a continuous availability of fish for angling during the 75-year recorded history of the fishery. Analysis by decades (Table 3), representing 30,062 fish retained during 7619 angling trips, reveals a mean

TABLE 3. Harvest statistics for smallmouth bass from Tadenac Lake between 1896 and 1966.

Period	No. of angling trips	No. of fish retained	Catch per angling trip
1896–1900	775	3,103	4.0
1901–1910	1,774	10,406	5.9
1911–1920	560	1,960	3.5
1921–1930	626	1,908	3.1
1931–1940	866	3,653	4.2
1941–1950	1,214	3,272	2.7
1951–1960	1,254	3,397	2.7
1961–1966	550	2,363	4.3
Total	7,619	30,062	
Annual	107	423	4.0

catch per trip of 3.9 (2.7–5.9) fish. Between 1963 and 1966, fishing rate was 1.1 (0.8–1.5) fish per rod-hour, which is similar to that recorded by Doan (1940) for Lake Opeongo (1.2–1.9) but greater than that recorded by Fraser (1955) for South Bay, Lake Huron (0.5–0.9).

Annual harvests in Tadenac Lake from 1963 to 1966 were between 0.2 and 0.4 k/ha (0.19–0.37 lb/acre) or about 4.0 k/ha (3.5 lb/acre) if only the littoral area inhabited by smallmouth bass during the summer months is included. Because of limited fishing pressure and an angler preference for fish over 30 cm (12 inches), more intensive angling would be expected to increase annual harvests substantially.

Conclusion — Observations on the Tadenac Lake fishery indicate that small lakes with controlled water levels, and offering suitable littoral habitat, may provide thermal and other environmental conditions suitable for natural reproduction and an acceptable growth rate of smallmouth bass even at latitudes near the northern limits of its native range. It would be expected, therefore, that the introduction of smallmouth bass into small lakes ecologically similar to Tadenac Lake would establish self-sustaining populations capable of providing either worthwhile local sport fisheries or stocks of young fish for transplant to other waters where satisfactory conditions for natural reproduction limit production.

Acknowledgments — The study was undertaken in cooperation with the Tadenac Club Limited, which made their facilities freely available and provided financial assistance.

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A Submersion–Emersion Sensor, for Intertidal Biological Studies[1]

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An instrument for use in intertidal biological studies that determines, at any instant, whether a given intertidal site is under or above water is described. The instrument measures microsubmersions and microemersions caused by surf.

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IN A STUDY evaluating environmental parameters responsible for intertidal zonation it was necessary to determine the actual periods of emersion and submersion of the biota. We could not use a tide gauge because of the unpredictable nature of surf and were unable to find any published account of an instrument that would provide us with the requisite information. The following is a description of a recording sensor that determines, at any instant, whether a given intertidal site is under or above water.

The sensor consists of two brass rods 10 cm long, 0.5 cm in diameter mounted horizontally 2.5 cm above a plexiglass plate, 1.3 cm thick, 10 × 14 cm (Fig. 1). The rods are connected by rubber-insulated telephone dropline to a battery-operated Rustrak model 91, 6-v dual channel recorder situated above high water. The distance between the rod ends is adjusted with wing-screws to assure maximum deflection of the recorder stylus when the sensor is submerged and to prevent retention of a water drop between the rod ends during periods of emersion. Sea water on the sensor completes an electrical current that in turn causes a deflection of the recorder stylus.

When in operation the sensor is attached to the substratum by a 14-mm bolt screwed through the base into an anchored Phillips shield 14 mm in diameter. A heavy rubber strip acts as a shock line between the Phillips shield

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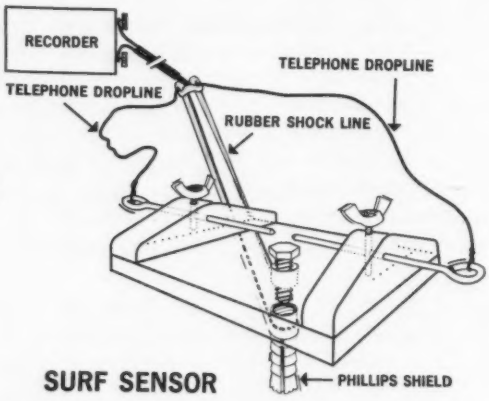

FIG. 1. Schematic design of submersion–emersion sensor and associate apparatus.

and telephone dropline, protecting the brass rods and wire connections from any force applied on the telephone dropline.

The type of data obtained from sensor recordings is shown in Fig. 2. Two sensors were positioned at the same vertical level, sensor A located on a more exposed substratum than sensor B. The recordings for the two sensors were made concurrently. When the tracing follows the base line the sensor is continuously emerged. Individual spikes indicate microsubmersions resulting from single waves. Confluent spikes result from the action of successive waves with short periods. Continuous submersion is indicated by a level tracing along the top of the chart. Comparative analysis of the 2-m environments can be made with respect to number, duration, and times of submersions and emersions. If tidal data are available, it is also possible to determine the relationship between theoretical and actual submersion–emersion histories of the environments (Green, MS, 1967).

The submersion–emersion sensor described above records the history of submersions and emersions to which intertidal organisms are subjected. One physical parameter, wave force, is not recorded but is of biological interest. Instruments for recording wave force are described in the literature (Jones and Demetropoulos, 1968; Muus, 1968) and these could be used in conjunction with the submersion–emersion sensor. The construction, installation, and operation of the submersion–emersion sensor is simple and inexpensive. It has been used successfully during periods when surf was greater than 3 m.

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(FIG. 2 *opposite*)

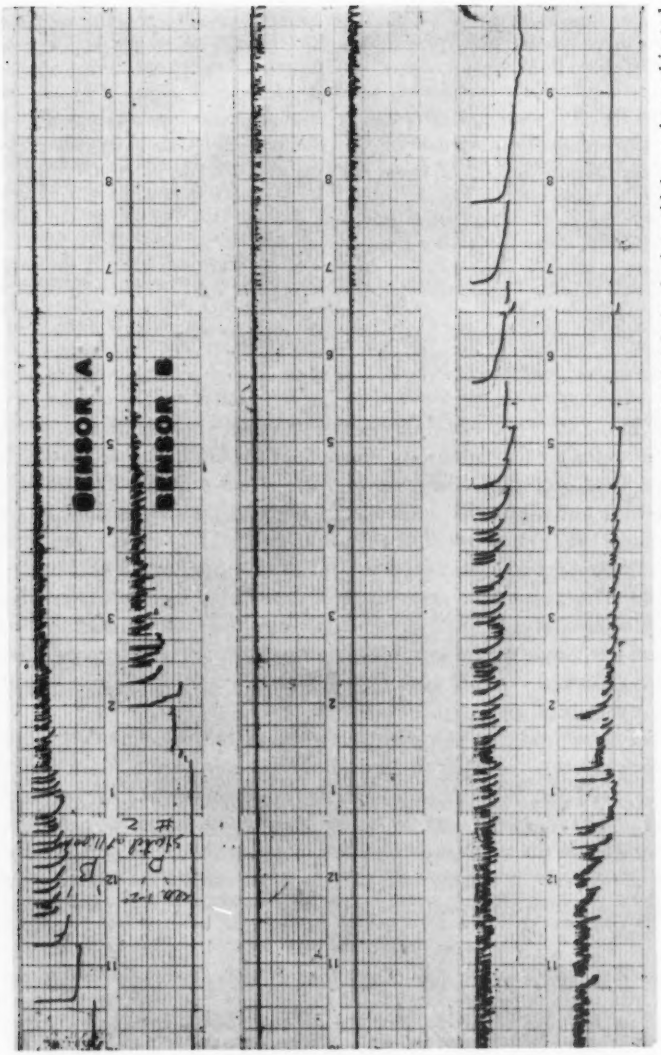

FIG. 2. Concurrent records from two sensors positioned at the same vertical height in the intertidal zone but subjected to different degrees of exposure. Each numbered chart division equals 10 min.

Effects of Temperature on Serum Protein Components in the Killifish, *Fundulus heteroclitus*[1]

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UMMINGER, B. L. 1970. Effects of temperature on serum protein components in the killifish, *Fundulus heteroclitus*. J. Fish. Res. Bd. Canada 27: 404–409.

Electrophoretic patterns of serum proteins were studied in *Fundulus heteroclitus* acclimated to temperatures from 30 to −1.5 C. Microzone electrophoresis on cellulose acetate revealed eight distinct zones (seven anodal and one cathodal to the origin) at all temperatures. These zones were grouped into three larger zones (I, II, and III) for convenience in analysis. Low mobility proteins of zone III decreased in the cold to a greater degree than did high mobility proteins of zones II and I. The greater reduction in these low mobility proteins, which are comparable to human gamma globulins, might produce a less active immune response in cold-acclimated killifish.

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STUDIES dealing with the effect of temperature on the electrophoretic pattern of serum proteins in fish generally show that low mobility fractions (usually gamma and beta globulins) decrease and that high mobility fractions (usually alpha globulins and albumins) increase during acclimation to the cold. Meisner and Hickman (1962) reported that the albumin/globulin ratio was significantly higher in rainbow trout acclimated to 8 C than in fish at 16 C. Poston (1966) found similar changes in the albumin/globulin ratio in brown trout. Likewise, Smirnova (1962) observed that in eight species of fish maintained in a reservoir and sampled with season, the serum albumins increased in winter and decreased in summer. Flemming (1958) also noted that the albumin/globulin ratio was altered in overwintering carp.

In a previous study on the effects of subzero temperatures on the killifish, *Fundulus heteroclitus*, total serum protein levels were often found to be unaffected by the cold. Occasionally they did show a decrease at lower temperatures (Umminger, 1970b). However, no attempt was made to look at the effect of cold acclimation on the electrophoretic pattern of the serum proteins. The purpose of the present investigation, then, was to extend the previous study by examining the effect of temperature on the electrophoretic pattern of the serum proteins.

Materials and methods — The vials of sera from *Fundulus heteroclitus* used in the present investigation were the same ones used for the determination of total serum protein in a previous study (Umminger, 1970b). To avoid confusion, the group numbers assigned to fish in the previous

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study have been employed to refer to those same groups of fish in the present investigation. The details of the procedure employed in handling, acclimating, and bleeding the fish can be obtained from a previous paper (Umminger, 1970a).

All fish were adult males that had been acclimated to a given temperature in sea water for at least 10 weeks before autopsy, except for fish kept at subzero temperatures. The period of acclimation for fish at subzero temperatures was only 1–2 weeks. At autopsy, free-flowing blood was collected from the caudal artery of the severed tails. Since the volume of serum collected from a single fish was small, pooled samples from two to five fish were collected by adding the required amount of serum, in sequence, to bring the volume to 100–150 μliters in graduated 250-μliter vials. When the number of pooled samples was sufficient, statistical treatment could be employed. Group 1 utilized 11 fish at 20 C to produce two pooled samples and 18 fish at 10 C to yield five pooled samples. Group 2 consisted of four pooled samples from 17 fish at 20 C and six pooled samples from 18 fish at 2 C. Group 3 utilized 10 fish each at 30, 20, and −1.5 C; only two pooled samples were obtained at each temperature. Group 4 consisted of four pooled samples from 24 fish at 20 C, four pooled samples from 19 fish at 10 C, five pooled samples from 10 fish at 2 C, and seven pooled samples from 15 fish at −1 C.

The serum protein patterns were determined with a Microzone Electrophoretic System (Beckman Instruments, Inc.) using a general protein stain (Ponceau S in 3% trichloroacetic acid). Procedures were similar to those described by Grunbaum et al. (1963) with minor modifications suggested by the Beckman Instructions Manual. It was not possible to compare individual zones in different protein patterns since distinct valleys were not always present. Therefore, the total protein within R_f ranges taken at obvious separation points was compared. The total protein pattern was divided into three zones, taking the cathodal to anodal boundaries of the spread as 0–1.0: zone III = 0–0.5, zone II = 0.5–0.8, and zone I = 0.8–1.0 (Fig. 1).

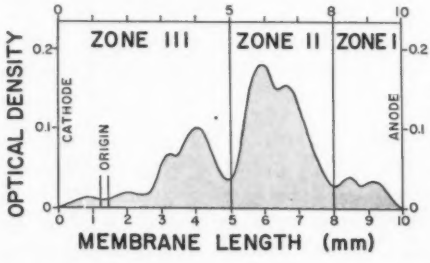

FIG. 1. Electrophoretic protidogram of a serum sample from *Fundulus heteroclitus* acclimated to 20 C, illustrating the arbitrary zones I, II, and III designated in the text.

The number of integrator units for each zone of the protein pattern was calculated from densitometer tracings of the cellulose acetate strips and expressed as the percentage of the total number of integrator units for the entire protein pattern. Since the absolute amount of total protein (in grams %) in each sample was known from previous studies (Umminger, 1970b), the absolute amount of protein in each of the three zones could be determined by multiplying the amount of total serum protein by the percentage of the total that was present in that particular zone. All data given in this paper are means ± standard errors calculated for small sample numbers and statistical significance was determined using Student's *t*-test.

Results — Microzone electrophoresis of serum on cellulose acetate revealed eight distinct protein zones (seven anodal and one cathodal to the origin; Fig. 1). As stated above, these eight zones were grouped into three larger zones for convenience in analysis.

Fish in experimental groups 2 and 4 revealed significant changes in the amount of protein in the various zones (Table 1). The other two groups of fish (1 and 3) did not have a sufficiently large sample size to permit statistical

TABLE 1. Total serum protein (g %) in electrophoretic zones I, II, and III. Data expressed as mean ± standard error (sample size).[a]

Group	Zone	Temp (C) 30	20	10	2	−1	−1.5
1	I	–	0.66 (0.62, 0.70)	0.77±0.06(4)	–	–	–
	II	–	1.82 (1.68, 1.96)	1.98±0.14(4)	–	–	–
	III	–	1.09 (0.91, 1.29)	0.98±0.08(4)	–	–	–
2	I	–	1.00±0.03(4)	–	0.54±0.046[b]	–	–
	II	–	1.99±0.10(4)	–	1.03±0.04(6)[b]	–	–
	III	–	1.60±0.07(4)	–	0.47±0.05(6)[b]	–	–
3	I	1.01 (0.91, 1.12)	0.93 (0.84, 1.02)	–	–	–	0.36 (0.36, 0.36)
	II	2.80 (2.73, 2.87)	2.35 (2.10, 2.59)	–	–	–	1.05 (0.88, 1.21)
	III	1.54 (1.26, 1.82)	1.45 (1.26, 1.64)	–	–	–	0.47 (0.43, 0.51)
4	I	–	0.63±0.02(4)	0.79±0.05(4)	0.79±0.05(5)	0.65±0.06(7)	–
	II	–	1.66±0.08(4)	1.98±0.06(4)	1.59±0.08(5)	1.88±0.09(7)	–
	III	–	0.96±0.07(4)	0.75±0.06(4)	0.67±0.04(5)[b]	0.63±0.06(7)[b]	–

[a]When sample size is two, the individual values are given in parentheses.
[b]Significantly different from 20 C controls ($P < 0.02$).

treatment of the results, but variations in the protein content of the zones with temperature were nevertheless evident. In fish of group 2 kept at 2 C, the serum protein decreased by 46% in zone I, by 48% in zone II, and by 70% in zone III. All decreases were significant and accounted for the 56% decrease in total serum protein observed in this group. In fish of group 4, the amount of protein in zone III dropped significantly by 30% at 2 C and by 34% at −1 C; the other two zones showed no significant changes with temperature. These data clearly show that proteins in zone III decreased in the cold to a greater extent than did the proteins in zones II and I. This can be seen from Table 2, which expresses the ratio of the sum of zone I and zone II proteins

TABLE 2. Ratio of the amount of serum protein in the fast-moving electrophoretic zones (I plus II) to the amount of protein in the zone of low mobility (III).

	Temp (*C*)					
Group	30	20	10	2	−1	−1.5
1	–	2.33	2.89	–	–	–
2	–	1.87	–	3.58	–	–
3	2.54	2.27	–	–	–	3.05
4	–	2.40	3.69	3.55	4.03	–

to zone III proteins (I + II/III). Since zone III proteins decreased to a greater extent in the cold, this ratio gets correspondingly larger in cold-acclimated fish.

Discussion — Pickford et al. (1969) showed that the low mobility proteins of zone III in the serum of *F. heteroclitus* were comparable to human gamma globulins. The proteins in zone II were found to be comparable to human beta globulins. The high mobility proteins of zone I had electrophoretic migration patterns similar to those of human alpha globulins. No serum albumin was present. Although it is not valid to deduce a similarity of function from a similarity in electrophoretic migration patterns, there is evidence that the low mobility proteins in fish serum function as do human gamma globulins in being antibodies. For example, Flemming (1958) observed an increase in the gamma globulin fraction of the serum proteins in acutely ill carp. Sorvachev et al. (1962) found that progeny of hyperimmunized carp had higher gamma globulin levels than those of nonimmunized fish. Post (1963, 1966) reported that specific antibodies in the sera of immunized rainbow trout were contained in the least mobile fraction of the serum proteins. Summerfelt (MS, 1964, 1966) found the same relationship in the golden shiner: antibody function was correlated with the protein fraction with lowest electrophoretic mobility and corresponded to human gamma globulin. Furthermore, Bouck and Ball (1967) observed that fish species with low levels of low mobility serum proteins were least likely to survive in polluted waters.

If the low mobility serum proteins of *F. heteroclitus* function as antibodies, as is the case for other fish, then the reduction of these proteins in the cold might be expected to cause an increased susceptibility to disease at low temperatures. Nothing is known about antibody production and immunity at low temperatures in *F. heteroclitus*. There is a large amount of literature dealing with the effects of temperature on antibody production and immunization in other fish. Temperatures of 10 C or above are generally required to produce rapid and vigorous immune responses in fish (Nybelin, 1935; Wollman, 1938; Pliszka, 1939; Cushing, 1942; Bisset, 1946, 1947, 1948a, b; Hildemann, 1957; Krantz et al., 1963). This retardation in the immune response of cold-acclimated fish is probably due to the lowered levels of serum gamma globulins demonstrated by electrophoresis. Therefore, the assumption can be made that the immune response is less active in cold-acclimated *F. heteroclitus* since the levels of low mobility serum proteins are reduced.

However, the groups of *F. heteroclitus* exposed to low temperatures were not more susceptible to disease than groups at warm temperatures. In fact, the outbreak of disease was often more common in warm- than in cold-acclimated fish. Cold temperatures probably preclude the need for the immune response in fish because pathogens are also at a low response level. There is less need for immune bodies in the blood at low temperatures because cold inhibits pathogen growth and reproduction.

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Hermaphroditism in a Pacific Hake, *Merluccius productus*, from Puget Sound, Washington

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MILLIKAN, A. E., AND B. H. PATTIE. 1970. Hermaphroditism in a Pacific hake, *Merluccius productus*, from Puget Sound, Washington. J. Fish. Res. Bd. Canada 27: 409–410.

An hermaphroditic Pacific hake is reported encountered during a routine biological examination of over 12,000 individuals. The normal right ovary was accompanied by the left ovotestis. The size and age of the fish suggests this condition did not adversely affect growth.

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HERMAPHRODITISM has been observed in many groups of fish including herring, sardines, salmonids, carps, cods, and tunas (Dawson, 1964, 1966). Although hermaphroditism is rare in most species, Morgan and Gerlach (1950) reported

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almost 3% of the striped bass, *Roccus saxatilus*, examined from Coos Bay, Oregon, were hermaphroditic, and protandric hermaphroditism is normal in some serranids (Lagler et al., 1962). Ciechomski and Christiansen (1968) observed an individual merluza (*Merluccius merluccius hubbsi*) with an obvious ovotestis, but apparently there is no published record of hermaphroditism in Pacific hake, *Merluccius productus*.

We are reporting the first observation of a bisexual Pacific hake encountered during routine biological examination of over 12,000 individuals landed from a reduction fishery in central Puget Sound, Washington. The specimen was taken from a vessel load of hake that was captured in Port Susan in April 1969 and held several days without benefit of ice or refrigeration. Only gross examination of the fish was possible because of its deteriorated condition.

The external appearance of the fish was normal. It was 42 cm fork length and 7 or 8 years old as determined by otolith interpretation. Preliminary examination revealed paired gonads, elongate, and joined posteriorly. The left gonad was an apparently normal ovary in an advancing stage of maturation with yolked yellow–orange ova. Very few ova had reached full maturity characterized by transparency and increased size. The right gonad was a distinct ovotestis with the anterior two-thirds an abnormal testicular mass that integrated posteriorly into normal ovarian tissue. The testis was comprised of many flat fingerlike protrusions rising from a large globular creamy-white mass and obviously distinct from the flat, convoluted testes of normal male hake. Microscopic examination of the abnormal testis revealed many malformed and misshapen spermatozoa. Very few normal spermatozoa were present.

It is doubtful that this hermaphroditic condition adversely affected the fish since both its age and length were much greater than the sample mean.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The boldface number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
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Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(4) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(5) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(6) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(7) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(8) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

Studies No.

1386. BARRACLOUGH, W. E., R. J. LEBRASSEUR, AND O. D. KENNEDY. 1969. Shallow scattering layer in the subarctic Pacific Ocean: detection by high-frequency echo sounder. Science 166: 611–613. (1)

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1389. EVELYN, T. P. T. 1968. Tissue levels of chloramphenicol attained in sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon by feeding. Bull. Off. Int. Epiz. 69: 1453–1463. (1)

1390. TANAKA, S., M. P. SHEPARD, AND H. T. BILTON. 1969. Origin of chum salmon (*Oncorhynchus keta*) in offshore waters of the North Pacific in 1956–1958 as determined from scale studies. Int. N. Pac. Fish. Comm. 26: 57–155. (1)

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1395. Ackman, R. G., and S. N. Hooper. 1970. Branched-chain fatty acids of four freshwater fish oils. Comp. Biochem. Physiol. 32: 117–125. (7)

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Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

EDITORIAL PRACTICES

An abstract must be provided in the same language as the text; authors are encouraged to provide both English and French versions.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. N.W., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopedique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Guide for Typists Preparing Copy for

Journal of the Fisheries Research Board of Canada

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables Examine the current volume of the *Journal* to see how tables are set up (Appendix C). Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

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Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

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Illustrations Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for Illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical expressions, equations, and formulas Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices Appendices A to F are available on request from the Office of the Editor.

MEMORANDUM

TO: Fishery scientists, researchers, librarians, students, teachers, etc.

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RE: Indexes to 70 years of FRBC publications, 1900-1969

The first index is Bulletin 164 published in 1968, entitled "Index and List of Titles, Fisheries Research Board of Canada and Associated Publications, 1900-1964," by Neal M. Carter. It contains 649 pages and sells for $4.00 from the Queen's Printer Bookshops listed below.

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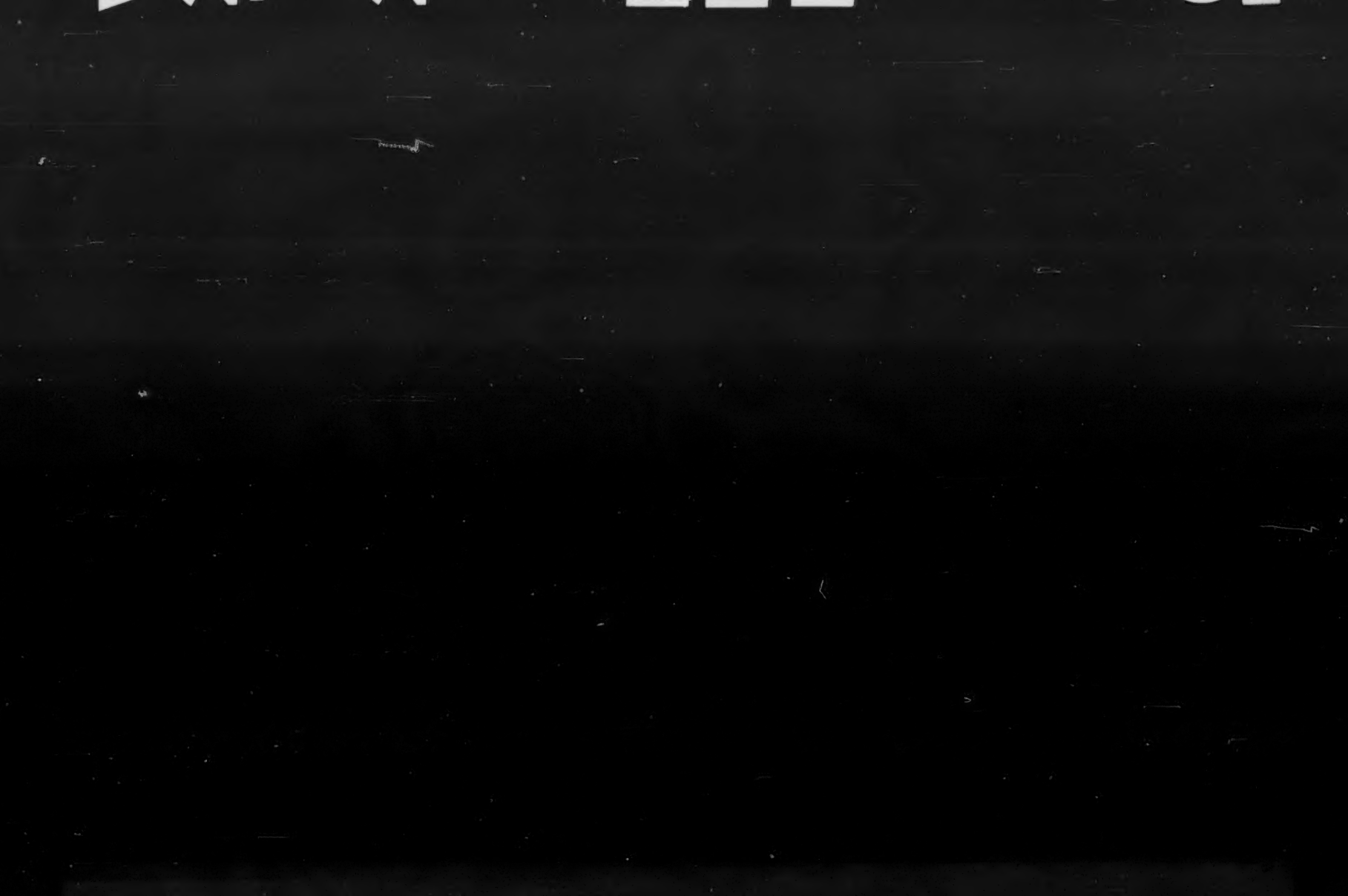
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